The Effect of Natural Alternative Sweeteners Lucuma, Yacon, and Monk Fruit on the Growth of Probiotic Lactic Acid Bacteria

A thesis submitted for the degree of

Masters by Research (MbR)

by

Elizabeth Kahler

School of Applied Sciences,

Abertay University

December, 2020

Declaration

Candidate's declarations:

I, Elizabeth Kahler, hereby certify that this thesis submitted in partial fulfilment of the requirements for the award of Masters by Research (MbR), Abertay University, is wholly my own work unless otherwise referenced or acknowledged. This work has not been submitted for any other qualification at any other academic institution.

Signed [candidates signature]..[redacted]……

Date……1/12/2020……..

Supervisor's declaration:

I, Boon Seang-Chu, hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Masters by Research in Abertay University and that the candidate is qualified to submit this thesis in application for that degree.

Signed [Principal Supervisors signature] ……[redacted]……..

Date……1/12/2020……..

Certificate of Approval

I certify that this is a true and accurate version of the thesis approved by the examiners, and that all relevant ordinance regulations have been fulfilled.

Supervisor……..

Date……..

Acknowledgements

I am quite fortunate to have had the guidance, knowledge, and support of such a great supervisory team, Boon, Sarah, and Athina, for the duration of this project. First and foremost, sincere appreciation goes to my principal supervisor Boon. His patience, guidance and continual positive encouragement through out the course of this work has allowed me to develop into a better scientist. My sincere thanks goes to the supporting staff of the graduate school, the food science department, and additionally the lab technicians for their assistance throughout the course of this work.

I am indebted to my family for their understanding and well wishes. My deepest gratitude goes to Mom, for I could not have done any of this without her unconditional love and understanding, continuous support and encouragement. I would like to express special appreciation to Steve and the Bond family. Due to your friendship, love, and support I am able to finish this vital goal of my life.

Abstract

Research continues to emerge indicating the potential destructive effects of non-nutritive sweeteners on the viability of gut microbe populations. Recent trends demonstrate an increase in demand for natural and plant-based replacements for artificial sweeteners. Yacon, lucuma, and monk fruit are novel ingredients that exhibit low calorie sweetening power in addition to multifunctional attributes that make them useful as alternatives to artificial sweetening substances. Fermentable sugars contained within these sweeteners have the potential to display prebiotic impact for active growth of beneficial probiotic bacteria. Lactic acid bacteria are a populous group of probiotic organisms with a long established history of consumption in fermented foods and an association with functional impacts on the structure of the gut microbiome. There is little research pertaining to the influence of sucrose substitutes on the viability of probiotic bacteria, variable specificity of the different strains, and how compatibility with different sweeteners plays a major role in their survival. In this study, we investigated the effect of novel sweeteners on the acidification of skimmed milk during fermentation with probiotic bacteria. In culture medium, acidification kinetics were inhibited in the presence of the sweeteners, suggesting that the sugars and/or other compounds contained in the sweeteners displayed mechanisms of inhibition which possibly suppressed growth of the lactic acid bacteria. However, there was no significant difference in viability ($p > 0.05$) between in treatments and control. This study was limited and experiments were cut short by the sudden lockdown due to the Covid-19 pandemic. It is suggested that future research employ chromatographic techniques to analyse the sugar composition of the sweeteners. Viability analysis experiments which yielded inconclusive data should be repeated. Additionally, variations in starter cultures may be explored for their specific enrichment of gut probiotic populations at the strain level. A sensory component will help inform new product development.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Ace-K potassium acesulfame

AGI α-glucosidase inhibitors

CLA conjugated linolenic acids

DP degree of polymerisation

DW dry weight

FF Functional food

FOS fructooligosaccharides

GI glycaemic index

GIT gastrointestinal tract

GM gut microbiome

GOS galactooligosaccharides

GRAS generally recognized as safe

IBD inflammatory bowel disease

IBS irritable bowel syndrome

LAB lactic acid bacteria

NNS non-nutritive sweeteners

SCFA short chain fatty acids

T2DM type 2 diabetes

1 Introduction

With the steady rapid increase of easily accessible and available options for high-calorie, low-nutrition foods, the rates of obesity have continued to rise to epidemic proportions. One way to reduce caloric intake is by reducing or eliminating the use of table sugar. In search of the latest promise of taste satisfaction along with a reduction in calories, the market for new foods continues to grow, and manufacturers have responded to this need by producing and commercializing synthetic sweeteners. Despite government approval and assurance of safety, consumers remain sceptical of the sweeteners, and there are concerns about their long-term safety (Suez *et al.*, 2014).

Research continues to emerge indicating that diet exerts a large effect on the gut microbiota (Donaldson *et al.*, 2015), thereby suggesting that modulation of our diet may have a significant impact on our health (Wang, 2009). The close relationship between diet, the gut microbiome, and health warrants further studies of more specifically what foods impact the microbiota. Artificial sweeteners have been observed to alter gut microbiome composition (Wang *et al.*, 2018). For examples, aspartame, sucralose, saccharin, and stevia altered the gut microbiota, leading to dysbiosis and the development of glucose intolerance in mice (Suez *et al.*, 2014). Some of the sweeteners have been shown to cause obesity and diabetes, an ironic result for something that is supposed to be a solution to these issues (Ruiz-Ojeda *et al.*, 2019).

Recent trends demonstrate the turn away from industrialized, processed food, and an increase in demand for natural, plant-based products as acceptable substitutes for artificial sweeteners. Functional foods (FF) are those that are considered to have an additional physiological benefit beyond basic nutritional requirements. Yacon, lucuma, and monk fruit may be suitable replacements for artificial sweeteners that may compromise health. These three natural sweeteners have certain associated health benefits and are gaining popularity with consumers in the U.S. Additionally, these sweeteners do not create a strong residual flavour in food preparations such as NNS are known to. The yacon tuber and the lucuma fruit have been used as traditional foods in South America for many years but the plants remain underutilized for their

potential as alternative sweetening ingredients in FF development. The Asian fruit Luo han guo ("monk fruit") has been traditionally used as a non-caloric sweetener (Zhang *et al.*, 2011). It is an accepted GRAS (Generally Recognized As Safe) ingredient that contains mogrosides, high intensity glycosides that are known to be 100-250 times sweeter than sucrose (USDA, 2017).

A prebiotic is a non-digestible fibre that escapes digestion in the small intestine and is readily fermented in the colon, making them available to be metabolized by gut microbiota, thus conferring benefit(s) upon host health (Gibson *et al.*, 2017). Dietary intake of fibres and prebiotics exerts a positive impact on probiotic viability and enhances the intestinal microbiota. Since prebiotics are naturally contained in fruits and vegetables, they offer a direct approach to manage the microflora through diet. Some sources of prebiotics also provide sweetening power and may be used as replacements for sugar or artificial sweeteners. These plants remain underutilized for their potential as alternative sweetening ingredients in FF development. A novel nutritional approach may be using these plant-based prebiotic ingredients to enhance foods that modulate a healthy gut microbiome and symbiotic host-microbial relationship.

1.1 Hypothesis/Aim

The present work aims to understand the impact of lucuma, yacon, and monk fruit sweeteners on the growth of co-culture *Lactobacillus delbrueckii* subsp*. bulgaricus* and *Streptococcus thermophilus* used in the production of yoghurt. Due to research indicating potential destructive effects of non-nutritive sweeteners (NNS) on the gut microbiome, we explored the viability of some relatively underutilized substitutes. It is hypothesised that since the sweeteners have prebiotic potential, it is postulated that they could enhance cell viability and growth when presented as a fermentation substrate for probiotic bacteria. Through this potential, the sweeteners could be possible ingredients for applications in FF development.

Another aim of this work was to conduct a narrative review analysing the findings of studies which examine the specific impact of NNS on the human gut microbiome and how this is associated with overall health or disease. While the

literature on the natural sweetener alternatives is somewhat limited, a comprehensive review was conducted to explore the chemical characteristics of the sweeteners and make an informed conclusion as to their effect on the microbiome, and potential as replacement sweeteners in food development. The chemical components of the sweeteners were investigated for their multifunctional characteristics, including medicinal properties, and as prebiotic agents with specific mechanisms that impact lactic acid bacteria and the gut microbiome as a whole.

Due to the Covid-19 pandemic, this dissertation is a hybrid of literature review and laboratory experiments. The objectives of this study are:

1. To conduct a narrative literature review of artificial sweeteners, natural sweetener alternatives, bioactive components, prebiotic impact on probiotic bacteria, and the gut microbiome, in order to establish familiarity with and an understanding of research in these areas;
2. To investigate the effect of lucuma, yacon and monk fruit sweeteners on the growth of yoghurt co-culture *Lactobacillus delbrueckii* subsp*. bulgaricus* and *Streptococcus thermophilus* in skimmed milk, by monitoring the change in acidification profile of the milk during fermentation and determining the cell viability in the end products;
3. To analyse the experimental data with appropriate statistical analysis.

2 Literature Review

2.1 Sugar, Artificial Sweeteners, and Health Concerns

2.1.1 Sugar

Despite early warnings of the impact of excessive sugar consumption on obesity and preventable health outcomes, global obesity rates have doubled over the last 20 years. Alongside the growing prevalence of obesity, the development of metabolic disorders such as type 2 diabetes (T2DM) is proportionally increasing. At the start of this century, the number of people globally with T2DM was estimated to be 171 million, and this number is expected to double by the year 2030 (Al-Goblan *et al.*, 2014). Besides these alarming figures is the social and economic costs incurred, therefore the urgent need to control this epidemic cannot be understated. The World Health Organization stresses that the limitation of nutritive sweeteners is an urgently necessary strategy to facilitate weight control and optimal nutrition (Gardner *et al.*, 2012). While the exact causes of diabetes are still not fully understood, maintenance of a normal body weight (body mass index < 30) is central to prevention and treatment of T2DM. Although all energy yielding food components have the potential to contribute to the positive energy balance that leads to weight gain, sugars are a huge source of hidden calories and provide no feeling of satiation, therefore a growing body of evidence indicates they are the major culprit in adverse health effects (Macgregor & Hashem, 2014). The correlation between sugar and development of obesity and T2DM is well accepted, and the desire to limit sugar intake has been a major driver of the popularity of non-nutritive substitutes.

2.1.2 Nonnutritive Sweeteners

NNS are a chemically diverse group of synthetic compounds that contribute an intensely sweet taste and are used to replace white sugar while containing little or no calories. They are categorised as high intensity sweeteners since they vary in power between several hundred and thousands times sweeter in reference to sucrose. Examples of NNS are aspartame, potassium acesulfame (ace-K), saccharin, and sucralose and they are commonly found in many beverages and convenience foods,

often without consumers realising their presence. NNS are commonly used for diabetics and weight loss diets, however, consumption of NNS containing foods has increased among people of all ages, with 28% of the total U.S. population reporting intake (Shankar *et al.*, 2013).

NNS are regulated by the Food and Drug Administration (FDA), European Food Safety Authority, and the World Health Organization. The FDA, which is a division of the U.S. Department of Agriculture (USDA) also publishes a Generally Recognized as Safe (GRAS) list of additives. Hundreds of toxicological and clinical studies are reviewed to make determination of safety, however the Dietary Guidelines Advisory Committee, a group of experts that publishes a scientific report for the USDA and Health and Human Services, cautions that added sugars should be reduced and not replaced with NNS (Sylvetsky & Rother, 2018).

The pathogenesis in the development of T2DM is based on the dysfunction of β-cells of the pancreas which causes a lack of control of blood glucose accompanied by insulin resistance. Diabetics are unable to control postprandial blood glucose without intervention. Because of the significant role of sugar consumption in the development of obesity and T2DM, the medical community has supported the use of NNS for overweight/obese and diabetic patients as a way of reducing the glycaemic index (GI) of foods (Fitch & Keim, 2012). The monitoring of carbohydrate intake and limitation of added sugars are the foundational strategies of the American Diabetes Association's recommendations for achieving glycaemic control. Evidence supports the notion that some carbohydrate sources are more favourable than others, depending on both their GI and fibre content, and there is a general consensus that diets low in GI are beneficial for the prevention and management of T2DM and other cardiometabolic diseases (Augustin *et al.*, 2015). The GI of a food represents the effect on postprandial blood glucose concentrations that the carbohydrate portion of a food has, compared with glucose or white bread. Low-GI carbohydrates, such as fructose, lower peak postprandial blood glucose and have been shown to have a positive effect on glucose control (Sheard *et al.*, 2004).

Although NNS use is now widespread, the topic continues to be controversial due to insufficient data and conflicting evidence related to the effects on metabolic disease. NNS are intended to prevent the morbidities that are known to be caused by

sugar, however some studies have demonstrated that their consumption is associated with adverse outcomes (Bright *et al.*, 2017). While some studies report an association between NSS use and reduced risk of obesity and T2DM (Wiebe *et al.*, 2011); other studies suggest that NNS alter metabolic parameters and promote the weight gain and glucose intolerance that can lead to T2DM (Feijó *et al.*, 2013). An intervention study associated NNS consumption with a reduction in energy intake and a decrease in body mass index, compared to a sucrose consuming group (Wiebe *et al.*, 2011). Contrary to this, in another study weight gain was promoted by the use of NNS versus sucrose, independent of caloric intake (Feijó *et al.*, 2013). There are concerns that NNS could offset any weight loss benefits, having an impact on appetite regulation that causes an ironic compensation in overall caloric intake in addition to an altered glycaemic response (Bruyère *et al.*, 2015).

It is thought that NNS may cause a shift in microbiota populations by inhibiting the survival of certain bacteria, though the findings have been inconsistent. Ace-K, or potassium salt of acesulfame, is about 200 times sweeter than sucrose and is metabolised in the body. One study that looked at the effects of ace-K on microbiota showed no significant differences in Bacteroidetes to Firmicutes ratio, or overall bacterial abundance (Frankenfeld *et al.,* 2015). Conversely, another study demonstrated ace-K to augment microbiota composition and exert a strong bacteriostatic effect on *E. coli* (Bian *et al.*, 2017a).

Sucralose is 600 times sweeter than sucrose and is derived from sucrose by the selective replacement of three hydroxyl groups by chlorine atoms. The sweetener is not digested, metabolised, or stored in the body (Magnuson *et al.,* 2016). Despite this finding, some research has shown sucralose to promote weight gain and metabolic disruption (Sylvetsky, 2018). It is suggested that there is an interaction with sweet taste receptors in the intestine that increases the rate of glucose absorption and insulin secretion from β-cells. The findings of one study revealed that the effects of sucralose on postprandial glucose metabolism can be differentiated between obese and normal weight participants, whereby higher plasma insulin concentrations were observed in the obese participants (Nichol *et al.*, 2019). Additionally evidenced in the literature is an altered gut microbiome whereby sucralose exerted a direct bacteriostatic effect on certain *E. coli* strains and promoted an increase in Firmicutes.

The mechanisms vary between species but are thought to either inhibit metabolic enzymes or alter nutrient transportation (Wang *et al.*, 2018).

Unlike most other NNS, aspartame is metabolised in the human body, breaking down into its components (phenylalanine, aspartic acid, and methanol) via enzymes in the digestive tract (Kroger *et al.*, 2006). Since the end products are absorbed without passing through the colon, it is thought they do not come into direct contact with the microbiota and are therefore metabolically inert (Magnuson *et al.*, 2016). Nevertheless, aspartame has been shown to cause dysbiosis of gut bacteria in mice, and a shift in microbial composition with an increase in *Clostridium leptum* and *Enterobacteriaceae* populations (Palmnäs *et al.*, 2014). Like sucralose, it is unclear what the mechanics are behind this effect and more research has been necessitated. Effects of NNS on the gut microbiome will be further explored in section 2.6.

Saccharin is the oldest NNS approved for food and beverage use in the United States (Fitch & Keim, 2012). Studies have related an increase in weight gain of mice consuming saccharin when compared to groups fed with sucrose, despite similar caloric intake (Feijó *et al.*, 2013). Consistent with this, another study showed that consumption of saccharin can promote excessive intake relative to glucose by reducing caloric compensation (Suez *et al.*, 2014). A plausible explanation for this is that the short-term caloric deficit leads to a lowered resting metabolic rate, which leads to an increase in long-term weight gain. Although using NNS may support the displacement of sugar in the diet, they may fail to compensate for satiety, possibly causing a higher overall caloric intake.

Research has demonstrated paradoxical associations with the consumption of NNS, demonstrating that they can help reduce added sugar intake in the diet and may support weight loss and diabetes management. Still, although the underlying mechanisms are compound specific, studies have made associations between artificial sweetener consumption and weight gain, impaired insulin sensitivity, metabolic syndrome, and a statistically significant increase in risk of T2DM (Anton *et al.*, 2010; Gardner *et al.*, 2012). These results raise the question of whether NNS are fuelling the epidemic they are intended to prevent (Fowler *et al.*, 2008). Consumers remain sceptical of NNS, and the scarcity of data regarding the safeness of these substances, leads to a great need for natural sweetener alternatives.

2.2 Natural Sweetener Alternatives

Along with the interest in sugar reduction, consumer preference for ingredients of natural origin has driven continued industrial focus on “clean” label formulations. Recent trends demonstrate the turn away from industrialised, processed food, and an increase in demand for natural, plant-based products as superior replacements for artificial sweeteners (van Gunst & Roodenburg, 2019). Due to the perceived risk of synthetic additives, consumers are willing to pay a premium for foods that are absent of these substances (Migliore *et al.*, 2018).

Among natural alternatives to sucrose and artificial sweeteners, and favoured due to their low glycaemic index are lucuma and yacon (Mérillon & Ramawat, 2018). Native populations of the Andean region have long cultivated these two crops, using both for diet and for traditional medicine (Fuentealba *et al.*, 2016). According to European Novel Food Regulation (258/97), safety approval is required for foods without a significant history of consumption within the European Union before 15 May 1997. Lucuma and yacon are foodstuff with sweetening properties and not food additives with an E-number classification, and not subject to the Novel Food Regulation (Hermann, 2009). The whole plants are typically dehydrated and milled into a flour and do not undergo any refining process, therefore may provide a high content of beneficial nutrients and bioactives (Belščak-Cvitanović *et al.*, 2015). A third sweetener which will be explored is monk fruit, which is in the category of high intensity non caloric sweeteners extracted from plants. In the United States, monk fruit extract was accepted as a generally recognized as safe (GRAS) ingredient in 2010, approved for its non-nutritive sweetening and flavour enhancing purpose (USDA, 2017). It is also approved for use in New Zealand, Australia, and Japan, though it has yet to be approved in the European Union (Younes *et al.*, 2019).

2.2.1 Lucuma

Figure 1
Lucuma Fruit and Powder

Lucuma (*Pouteria obovata*) is from a subtropical fruit tree belonging to the Sapotaceae family. The lucuma fruit is native to the temperate highlands of the Peruvian Andes and its use dates back to the Inca civilization (Duarte & Paull, 2015). Although Peru is the main producer, cultivars are also grown in highlands of Ecuador, Chile, California (United States), Mexico, Bolivia and Costa Rica. The fruits are divided into two varieties based on the consistency of their pulp: lucuma-seda with a softer flesh that is more appropriate to be eaten fresh, and lucuma-palo with a dense flesh that is consumed as a dried form (Gómez-Maqueo *et al.*, 2020). Lucuma fruit is round or ovaloid, green with a deep yellow-orange coloured flesh that is indicative of its high carotenoid content *(Figure 1).* Ripe fruits are dehydrated and milled into a mealy flour to extend storage, and the highly pigmented and somewhat dry pulp has a texture similar to pumpkin (National Research Council, 2000). Flour is prepared by selecting, disinfecting, peeling and seeding the fruit then drying at 60°C in hot air tunnels (Yahia, 2011). With a distinctive flavour that has a sweet taste that resembling caramel, butterscotch, or maple, the fruit could be used to flavour beverages, preserves, pudding, yogurt, ice cream, cake and cookie fillings, and other desserts (Ma *et al.*, 2004).

Fruits of the Sapotaceae family have been established as a rich source of novel anti-inflammatory polyphenols and antioxidant compounds (Brizzolari *et al.*, 2019). One study determined lucuma to have the highest concentration of phenolic

compounds among other Peruvian fruits (Silva *et al.*, 2009). Lucuma fruits are a good source of calcium, iron, niacin, and vitamin C, and the flour is very high in total dietary fibre (which is mainly in the insoluble form). The cultivar most commonly used for commercial production of flour is the Palo variety, which was shown to contain 31.7% insoluble fibre, and this is noted to be much higher than in other fruits such as pineapple, mango, or papaya. For a fruit, lucuma are significantly high in protein, with 1.5g - 2.4g per 100g of fresh pulp and up to 4g in the flour. They are low in acidity and high in reducing sugars (Glorio *et al.*,, 2008). Sugar content varies greatly in both biotype and maturity of the fruit, with unripe fruit containing primarily sucrose and sugar conversion leads to increased amounts of glucose and fructose throughout ripening stage. The sugars present in ripe lucuma fruit in order of highest to lowest abundance are glucose, fructose, sucrose and inositol (Yahia, 2011). In three different biotypes analysed for sugar content at commercial ripeness, fructose ranged between 18.8 ± 4.2 and 127.1 ± 34.9 mg/g DW (dry weight), glucose between 24.8 ± 7.0 and 173.3 ± 65.9 mg/g DW, and sucrose between 41.2 ± 13.8 and 77.5 ± 22.5 mg/g DW (Fuentealba *et al.*, 2016).

To control diabetes, it is important to regulate insulin sensitivity and postprandial blood glucose level. α-glucosidase is a digestive enzyme that participates in glucose digestion, and inhibition of this enzyme delays the degradation of starches to glucose. Agents with intestinal α-glucosidase inhibitory activity have been useful as oral α-glucosidase inhibitors (AGI), which play a major role in glycaemic regulation. A recent study found aqueous extracts of lucuma to behave as an AGI, producing a hypoglycaemic effect and attenuating blood glucose level, suggesting that lucuma may be a food-based treatment to complement diabetes management (Silva *et al.*, 2009). Because of the fruit's rich bioactive compound content and antihyperglycaemic effect, lucuma could be an underutilised ingredient with great potential for innovation and development of functional foods.

2.2.2 Yacon

Figure 2
Yacon Tuber and Powder

Yacon *(Smallanthus sonchifolius)* is a tuberous root which originates from the Andean region of South America, however it is now cultivated in several countries in the EU and Asia. Like lucuma, it is said to have been used as long ago as pre-Inca empire (Cao *et al.*, 2018). Yacon root may be brown, pink, purple or cream coloured and they bear a resemblance to sweet potato, however they lack starch and have a crunchy texture that is more like a radish or apple, with a juicy sweet flavour akin to watermelon or pear *(Figure 2).* The fresh tuber is primarily consumed raw, and the pulp is commercially produced into a dried flour in addition to an unrefined syrup which has a molasses like flavour and is made by concentrating the juice (Padilha *et al.*, 2017). Yacon root is a minor source of calcium, iron, and vitamin C, with a significant level of potassium.

Sweet taste can be attributed to the high fructooligosaccharides (FOS) content, of which the tuberous roots of yacon are considered to be one of the best natural sources. FOS are inulin-type fructans that consist of short chain fructose units linked by (2→1) β-glucosidic bonds with a terminal molecule of glucose (*Figure 3*). These bonds resist the hydrolysis of enzymes and digestion in the small intestine, allowing them to reach the colon microbiota. Yacon is a member of the Asteraceae family which also includes chicory and Jerusalem artichoke, which all contain the same major FOS (kestose, nystose and 1-fructofuranosyl nystose), but each of the sources of fructans exhibit a different degree of polymerisation (DP) and linkages between

adjacent fructose units. Yacon is noted to contain the highest concentration of fructans with a low molecular weight and low DP (Choque Delgado *et al.,* 2013). Chemical composition in yacon tubers may vary considerably depending on factors including genotype variation, planting location, growing season, harvest time, and post-harvest temperature. FOS have been cited to make up the bulk of total carbohydrates in yacon, varying between 40% and 70% of dry weight. Carbohydrate content is especially affected by post-harvest storage time and a traditional process of sunlight exposure that dehydrates the roots and accelerates the depolymerisation of FOS into sucrose and reducing sugars (glucose and fructose) (Graefe *et al.*, 2004).

FOS have about half the calories per gram than sucrose or glucose, with a sweetness of 0.3–0.6 relative to sucrose (Mérillon & Ramawat, 2018). The glucosidic bonds of FOS cannot be hydrolysed thus are not metabolised in the human digestive tract and several studies have shown that their consumption does not augment blood glucose levels. More human clinical trials are needed however several studies have associated health benefits with FOS consumption, including weight management and obesity prevention in overweight adults (Yan *et al.*, 2019). A 12 week trial looked at the effects of FOS supplementation on body weight in healthy adult subjects with a body mass index (BMI) >25. The study obtained results of a reduction in body weight of 1.03 ± 0.43 kg in healthy adults compared to a non-FOS consuming control group which gained 0.45 ± 0.31 kg (Slavin, 2013). Yacon is already acknowledged as a medicinal plant in its native regions, and commonly used as a remedy for diabetes management. Yacon roots are a good source phenolic and antioxidant compounds (Campos *et al.*, 2012). The bioactivity of yacon is further discussed later in this document. Yacon perhaps remains underutilised and could be well positioned as an alternative sweetening ingredient in FF development.

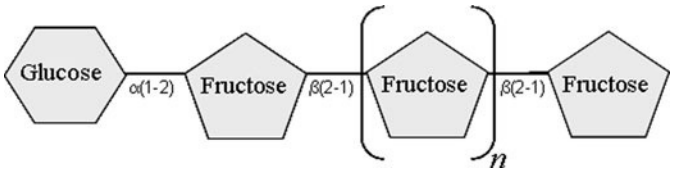

Figure 3
Fructan molecule (de Almeida Paula & Abranches, 2015).

2.2.3 Monk Fruit

Figure 4
Monk fruit and powder

Monk fruit (*Siraitia grosvenorii*), also known as luo han guo *(Figure 4),* is grown on a perennial vine indigenous to Asia, where it has been utilised for over 200 years (Zhang *et al.*, 2011). The main component is mogroside, which are cucurbitane-type triterpene glycosides reported to be 100-250 times sweeter than sucrose (Itkin *et al.*, 2016). Whole monk fruit contains sugars including fructose and glucose, but monk fruit extract are isolated mogrosides obtained by a process of water extraction, filtration, and selective concentration of the sweet glycosides. Depending on the manufacturing process, commercially available monk fruit products have varying concentrations of mogroside V, which is the main mogroside (*Figure 5*). The remainder of the product may be comprised of any combination of other terpene mogrosides, protein fragments, melanoidins, or flavonoids (Quinlan & Zhou, 2017).

In addition to its use as a natural sweetener, monk fruit is highly valued in traditional Chinese Medicine as a useful remedy for ailments which require cooling properties, such as sore throats, coughs (Li *et al.*, 2014). More recently, it has been researched for the pharmacological potential of mogrosides to serve as an agent with antidiabetic, anticarcinogenic, antibacterial and antioxidant properties (Suzuki *et al.*, 2007). Mogroside V may also be valuable as a chemopreventive agent, due to its demonstrated inhibitory effects on the initiation and progression of cancer (Lu *et al.*, 2012).

Mogrosides confer the sweet taste without increasing blood glucose (Pandey & Chauhan, 2019). *In vitro* evaluation of antioxidant capacity in mogroside V revealed strong oxygen free-radical scavenging activity, indicating an ability to counteract the oxidative stress induced by diabetes (Suzuki *et al.*, 2007). In addition, studies have indicated that the administration of the extract may contribute to the prevention of diabetic complications associated with oxidative stress and hyperlipidaemia (Qi *et al.*, 2008). In studies of diabetic rats that ingested maltose, mogrosides were shown to strongly supress rising postprandial blood glucose levels, exhibiting the potential to attenuate early clinical symptoms of T2DM (Jin & Lee, 2012). Mogrosides are a botanically derived AGI, inhibiting postprandial glucose peaks, thereby decreasing post-load insulin levels (Ghosh & Collier, 2012). Monk fruit's suitability as a plant derived sweetener, in addition to its role in disease prevention, indicates that it may be an invaluable natural resource in the functional food industry (Pandey & Chauhan, 2019).

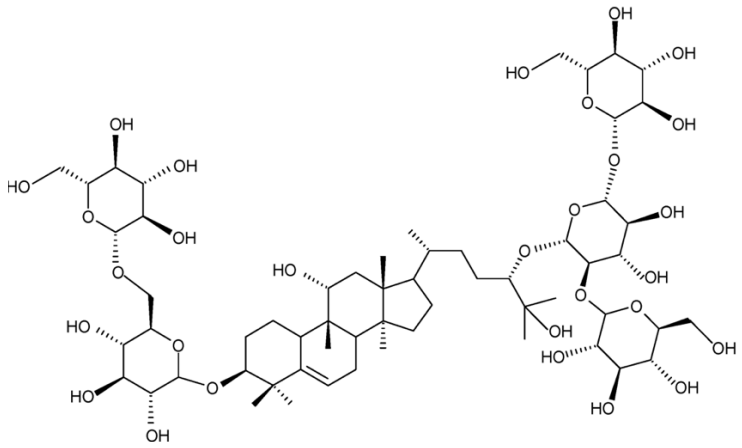

Figure 5
Chemical Structure of Mogroside V (Zhang *et al.*, 2011).

2.3 Bioactive Components

Many plants have been historically considered to be medicinal substances and can be utilised as nutraceuticals in the modern age. Fruits and vegetables contribute the protective health promoting factors and biologically active (bioactive) compounds which act in reducing the oxidative stress that leads to cellular damage. Plants contain

a heterogeneous collection of bioactive compounds which includes fermentable fibres, in addition to many antioxidant compounds including phenolic compounds, carotenoids, anthocyanins, coumarins, tannins, and tocopherols. Plant phenolic compounds (phenolics) are a diversified group of phytochemicals which includes a large subsection of chemicals called polyphenols, which can be further classified into lignans, phenolic acids, stilbenes, and flavonoids (*Figure 6*). The most widely distributed group, flavonoids are a family of phenolic compounds based upon the characteristic phenol structure of a benzene ring with at least one hydroxyl group bound to one or more aromatic rings. The various classes are defined by differing position and placement of functional groups on the carbon backbone (Baião *et al.*, 2017). They occur as glycosides (glucosides, galactosides, arabinosides, xylosides, and rhamnosides) which are stored bound to sugar moieties within the plant, requiring the sugar group (glycone) to be split from the polyphenol (aglycone) to render them more bioavailable. The absorption of dietary flavonoids liberated from food by chewing depends on their physicochemical properties such as molecular size and structure, configuration, and solubility. The majority of flavonoid glycosides do not meet the substrate specificity of digestive enzymes in the upper intestine and continue their journey to the colon, where they are hydrolysed and converted into their aglycan form by bacteria (Kumar & Pandey, 2013). Flavonoids possess strong antioxidant activity that is known to prevent the cell membrane oxidation which has been linked to the onset of numerous chronic diseases including cardiovascular (atherosclerosis, coronary heart disease, and heart attacks), neurodegenerative (Parkinson's and Alzheimer's), diabetes mellitus, cancer and inflammation (Ribas-Agustí *et al.*, 2018). As dietary components in humans, flavonoids are protective due to their high antioxidant capacity (Kumar & Pandey, 2013).

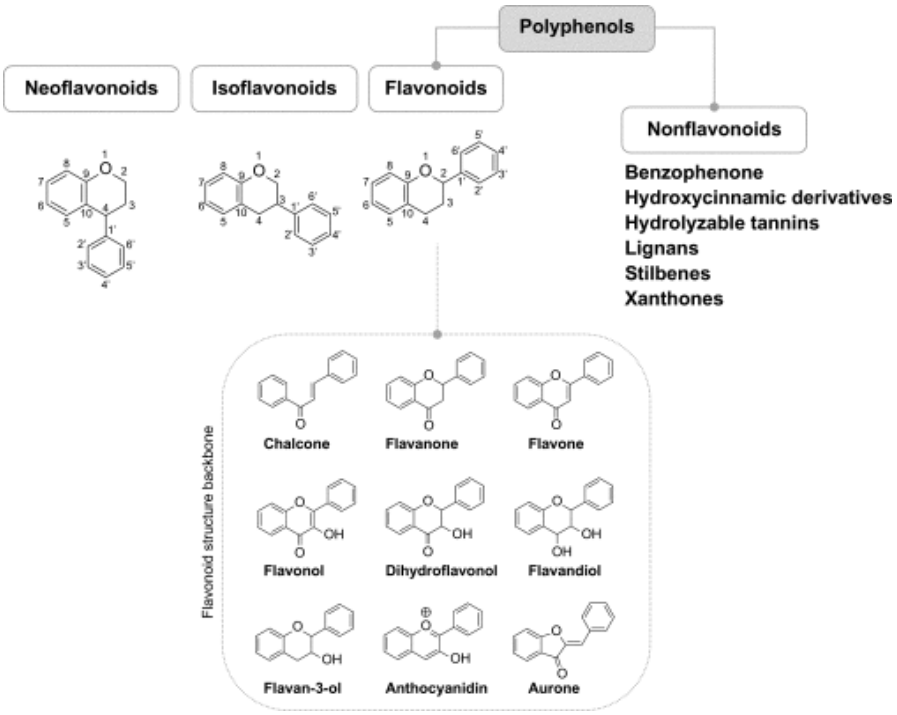

Figure 6

Chemical Classification of Polyphenols (Piccolella & Pacifico, 2015).

In food, phenolics may contribute to the bitterness, astringency, flavour, odour, colour, and oxidative stability. Food matrix composition has a great influence when evaluating bioaccessibility and bioavailability of phenolic compounds. In order to be bioavailable and render preventative health benefits, dietary phenolic compounds must be either released from the food matrices during small intestinal digestion (bioaccessible) or as a result of the transformative metabolism of dietary fibres in the colon, in which microbiota play a crucial role (Bohn, 2014). In a western diet, it is estimated that about 50% of phenolic compound intake is associated with dietary fibre. Diets that are abundant in plant foods have demonstrated the promotion of a stable GM profile, which may be due – at least partially – to the presence of fibre and polyphenols. Indeed, dietary fibre and polyphenol intake is consistently associated with increases in the growth of beneficial bacteria *Bifidobacterium* and *Lactobacillus* and other lactic acid bacteria which yield metabolites that provide antioxidant and anti-inflammatory effects (Singh *et al.*, 2017).

2.3.1 Phenolic Compounds in Natural Sweetener Alternatives

The sweeteners that are the focus of this work were found to contain various phenolic compounds, which elevates their dietary value and possible applications in nutraceuticals (*Table 1*). Yacon tubers are multifunctional foods that not only contain an abundance of health promoting FOS but also are rich in bioactive compounds such as phenolics up to 3.8% on a dry weight basis, although can significantly vary according to cultivar, environmental conditions during cultivation, post-harvest, and processing conditions (Campos *et al.*, 2012). Recent research has identified 25 bioactive compounds in yacon, such as tryptophan and caffeic acid. Tryptophan, an essential amino acid important to intestinal microbiota and brain function, exerts a positive effect on carbohydrate metabolism in hepatocytes due to increased activity of key enzymes (glycokinase, hexokinase, and glucose-6-phosphate dehydrogenase). Chlorogenic acid is the most abundant hydroxycinnamic acid in yacon and was shown to modulate plasma insulin concentration and inhibit of hepatic gluconeogenesis (Gomes Da Silva *et al.*, 2017). The antidiabetic effects of yacon root hydroalcoholic extract in streptozotocin-induced diabetic rats have been attributed to its antioxidant activity and hypoglycaemic effect of important phenolic compounds, mainly chlorogenic acid (Choque Delgado *et al.*, 2013). Besides the flesh processed into sweetener, some of the bioactives are found in other portions of the plant such as the leaves, stems, and flowers, either in higher amounts or exclusively. While the peel portion of yacon frequently contains a higher bioactive content, it also has a higher concentration of oxidation catalysts, and must therefore be completely removed in commercial processing (Manrique *et al.*, 2005). Yacon flesh rapidly turns brown once the root is peeled, therefore agents such as ascorbic acid, and cysteine are used to control the main oxidising enzyme, polyphenol oxidase. The distribution of sugars in the yacon root is such that the concentration of sugars increases from the centre to surface, and for this reason care must be taken in peeling since the highest FOS content is located right beneath the skin (Manrique *et al.*, 2005).

Other fruits of the Sapotaceae family have been the focus of most research and studies that characterise and quantify the bioactive metabolites in lucuma have been limited until very recently (Fuentealba *et al.*, 2016). Lucuma powder was reported to

contain a higher amount of phenolics (51.1 mg GAE/1000 g) than dehydrated fruits usually used in snacks, such as apples (14.30 mg/1000 g), blueberries (14.30 mg/1000 g), peaches (9.69 mg/1000 g), and strawberries (34.71 mg/1000 g) (Dini, 2011). Total Phenolic content (TPC) was shown to decrease as lucuma ripens, and the ripeness stage was shown to greatly impact levels of primary and secondary metabolites such as sugars, organic acids, and carotenoids. Total carotenoid content in lucuma was reported to be between 9-20mg / 100gFW, a high value considering carrots have carotenoid value between 10-40mg / 100gFW. There are many carotenoids besides β-carotene in lucuma which still require identification and quantification (Fuentealba *et al.*, 2016). A recent study noted that anti-hyperglycaemic effect in lucuma is related to the high concentration of triterpenoid α-amyrin, which supports glycaemic control due to α-glucosidase inhibitory activity (García-Ríos *et al.*, 2020).

Several important bioactive components that possess broad pharmacological properties have been isolated from monk fruit such as triterpenoids, flavonoids, and amino acids. Mogrosides are the main bioactive components in monk fruit, and currently over 60 types of mogrosides have been identified (Wang *et al.*, 2019b). The major flavonoid component of monk fruit is grosvenorine, which is metabolised by gut microbes producing metabolites with potent antibacterial action. In the early stage of immature fruit, Mogroside II is predominant; while in the later stages of maturity, more glycosylated sweet mogrosides (V) develop and accumulate (Wang *et al.*, 2019b). In extracts, the amount of mogroside is highly variable depending on the extraction, purification and concentration process, with concentrations of mogroside V varying from 25 to 55% of the crude extract (Younes *et al.*, 2019). There are a few significant biological aspects of mogrosides that evidence monk fruit's value as a medicinal food. Mogroside extract inhibited histamine release from mast cells in mice demonstrating its antihistamine properties, antioxidant activity was indicated through the effective elimination of free radicals, and inhibition of cancer cell promotion and progression suggests the capacity to act as a chemopreventative agent (Gong *et al.*, 2019; Takasaki *et al.*, 2003).

Table 1: *Bioactives in Lucuma, Yacon, and Monk Fruit*

Sweetener	Bioactive	Amount	Note	Reference
Lucuma	Triterpenoids (α-amyrin)	12.34 µg/g DW	Fruit extract (Seda biotype)	García-Ríos *et al.*, 2020
	Tocopherols (α-tocopherol)	59.4 ±1.3 µg/g DW	Fruit extract (Seda biotype)	García-Ríos *et al.*, 2020
	Phytosterols (Sitosterol) (Cycloartenol)	 5.27 µg/g DW 3.50 µg/g DW	Fruit extract (Seda biotype) (Beltrán biotype)	García-Ríos *et al.*, 2020
	Flavonoids (Dihydroflavanol Glycosides & Gallic Acid)	153.2 ± 3.5mg/ 100 g	Fruit extract (flour)	Dini, 2011
	Total Phenolic compounds (TPC)	2.50 ± 0.11 mg AGE/ g DW 5.11 ± 1.41mg AGE/ g DW	Fruit extract Dehydrated fruit flour	García-Ríos *et al.*, 2020 Dini, 2011
	Polyphenolic Antioxidants (Catechins & Gallocatechins)	304.6 ± 79.4 µmol TEAC /g DW	Aqueous extract from fruit at commercial ripeness (Rosalia biotype)	Fuentealba *et al.*, 2016
	Total Carotenoids (TC) Carotenoids (Hydrocarbon carotenes and Xanthophyll esters	131.6 ± 2 µg β-carotene /g DW 3944 ± 243 µg/ 100g FW 5197 ± 280 µg/ 100g FW	Ripeness stage 1 (Leiva biotype) Unripe fruit Ripe fruit	Fuentealba *et al.*, 2016 Gómez-Maqueo *et al.*, 2020
	Organic acids (Tartaric, Malic, Succinic, Quinic)	30.0-44.4 mg/ g DW	Aqueous extract from fruit (Rosalia biotype)	Fuentealba *et al.*, 2016
Yacon Root	β-(2→1) fructooligosaccharides (FOS)	58-78% of total DW 3-19% of total FW	Dry flour Fresh roots	Caetano *et al.*, 2016 Cao, Y *et al.*, 2018
	Total Antioxidants	23-136 µmol Trolox eq /g DW	Dry roots	Caetano *et al.*, 2016
	Total Phenolic Content	200mg /100g	Edible fresh matter	Yan *et al.*, 2019
	Phenolic acids (Chlorogenic acid) (Caffeic acid) (Ferulic acid, Galic acid)	7.9 ± 0.8 to 30 8 ± 0.1 39.1 mg/kg DW	Peel, flesh, root extracts Fresh tuber flesh Leaves and tuber	Simonovska *et al.* 2016 Pereira *et al.*, 2016
	L-tryptophan	0.5-2.8 mg/100g	Peel and Flesh	Campos *et al.*, 2012
	Flavonoids (Kaempherol, Myricetin, Rutin)	3222mg-4959 37mg/100g DW (DW=10.2-16.9g/100g FW)	Leaves and Flowers	Khajehei *et al.* 2018
Monk Fruit	Total Glycosides (mostly mogrosides)	1.19 mg/ 100 g	Fruit	Pandey and Chauhan, 2019

Total Flavones	5-10 mg/ 100 g	Fruit	Pandey and Chauhan, 2019
Major Mogrosides (Mogroside V is primary, followed by IV & VI)	0.55-65% fresh fruit (2 5% dried fruit)	Fruit	Jin and Lee, 2012 Younes *et al.*, 2019
Siraitiflavandiol	N/A	Ripe fruit	Jin and Lee, 2012
Kaempferol glycosides	N/A	Unripe fruit	Jin and Lee, 2012
Cucurbitane triterpenes Siraitic acid F and C	N/A	Roots	Jin and Lee, 2012
Triterpenoids (β-amyrin)	N/A	Vine and leaves	Jin and Lee, 2012
Antioxidants	47.396 ± 1.946 µg TEAC /mg	Aqueous fruit extract	Wuttisin and Boonsook, 2019
Total phenolic content	2.387 ± 0.063 mg AGE /mg	Ethanol fruit extract	Wuttisin and Boonsook, 2019
Total flavonoid content	25.229 ± 0 904 µg QE/mg	Aqueous fruit extract	Wuttisin and Boonsook, 2019

DW=dry weight, FW=fresh weight, AGE=mg of Gallic equivalents, TEAC=Trolox equivalent antioxidant capacity, QE=quercetin equivalents

2.3.2 Antimicrobial Activity of Polyphenols

Flavonoids are known to be synthesised by plants in response to microbial infection, and the inhibition of pathogenic bacteria (such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) exhibited by intestinal barrier protectors (several species of *Lactobacilli* and *Bifidobacteria*) is also attributed to the intake of dietary polyphenols (Saura-Calixto, 2011). These species are able to metabolise polyphenols and behave as antimicrobial agents via the inactivation of adhesins and enzymes and the disruption of microbial membranes (Kumar & Pandey, 2013). Antibacterial and antifungal activities make natural flavonoids act as antimicrobial food pr(Dini, 2011)(Dini, 2011). Phenolics present in roots and tubers such as methanolic extracts showed antibacterial activity against *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, *Salmonella typhi*, *Candida albicans*, and *Penicillium chrysogenum* (Chandrasekara & Kumar, 2016).

Flavonoids and their metabolites can shape gut microbiota by inhibiting the growth of various pathogens and by increasing beneficial genera such as *Lactobacillus*, which in turn improve gut health by reducing endotoxin production, promoting

nutrient absorption, and maintaining gut immune homeostasis. Microbiota metabolise flavonoids and create phenolic intermediates. Some polyphenols are catabolised to unique products such as flavan-3-ols and ellagitannins, which have beneficial functions in the gut such as the modulation of gene expression in colon cancer cells (Pei *et al.*, 2020).

2.4 Probiotic Bacteria

Probiotics are defined as living microorganisms that confer a health benefit on the host when administered in adequate amounts (Hill *et al.*, 2014). The International Scientific Association of Probiotics and Prebiotics (ISAPP) established this evolving concept regarding the strain specificity of probiotic effects: different strains express different mechanisms, although at times common mechanisms may be shared amongst strains of a larger taxo (Sanders *et al.*, 2018). Probiotic bacteria can be delivered through food sources or dietary supplements, including fermented dairy such as yoghurt or kefir (Kumar *et al.*, 2015).

2.4.1 Lactic Acid Bacteria

One of the most significant groups of probiotic organisms is Lactic acid bacteria (LAB). They are a diverse group of Gram-positive, non-spore forming, acid tolerant bacteria which are all dependent on the presence of fermentable sugars for active growth. A total of 16 genera make up the LAB group, with *Lactobacillus* as the largest genus, and 12 genera are used in food. Lactic acid is the major end product of sugar fermentation, and LAB may be either homofermentative, producing more than 85% lactic acid, or heterofermentative, producing lactic acid in addition to CO_2, ethanol (and/or acetic acid) in equimo(Holzapfel, 1995)(Holzapfel, 1995). Through their fermentation of carbohydrates, LAB provide for a great enhancement of flavour and texture in food products, in this way contributing to a pleasant sensory profile of the end product (Narvhus & Axelsson, 2003). Specific strains create inhibitory effects on certain pathogenic microorganisms, but LAB can also limit the invasion of pathogenic bacteria by multifactorial means, which involve the production of different inhibitory compounds and competitive exclusion for binding sites on the gut epithelium and for

nutrients. The exclusion may be attributed to the formation of biofilms which render pathogens unable to adhere to the intestinal epithelial mucosa, thereby reducing colonisation and preventing infection (Wedajo, 2015). LAB also produce bacteriocins, antimicrobials and organic acids such as lactic acid, acetic acid, and formic acid, which can be applied to prevent spoilage and to inhibit foodborne pathogens such as *Clostridium botulinum, Staphylococcus aureus,* and *Listeria mo*(Leroy & De Vuyst, 2004)(Leroy & De Vuyst, 2004). The fortification of intestinal flora components to increase gut resistance to pathogenic invasion may be a rational way to support prevention or treatment of gut related conditions such as peptic ulcers, ulcerative colitis, and *Candida* overgrowth, in addition to chronic gut disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and colon cancer (Manning & Gibson, 2004).

2.4.1.1 Probiotic Effect of Lactic Acid Bacteria

Beneficial association of lactic acid producing microorganisms with the human host and dates back to studies of gut ecology at the turn of the twentieth century. Elie Metchnikoff, regarded as the father of modern probiotics, is credited with introducing lactobacilli as probiotics to promote health, however *Lactobacillus bulgaricum* was named after the physician, Stamen Grigorov, who first identified the LAB in Bulgarian yoghurt (Walter, 2008). To this day the health improving mechanisms are still not fully understood but are suggested to relate to pathogen interference, exclusion or antagonism, modulation of the immune system, anticarcinogenic activity, amelioration of lactose intolerance, lesser diarrheal incidence and duration, and prevention of urinary and vaginal infections (Wedajo, 2015). LAB transiently colonise the human gastrointestinal tract (GIT) but some LAB strains can survive and colonise the gut longer term, having the capacity to alter the composition of the gut microbiota through their production of beneficial fermentation-derived metabolites (Wang *et al.*, 2017). *Bifidobacterium* and *Lactobacillus* produce organic acids known as short chain fatty acids (SCFA) in the colon via the fermentative pathway. Carbohydrates are metabolised into SCFA such as lactate and acetate, providing a range of potential benefits to the intestinal tract and beyond. LAB also play an important role of interaction with cells on the gut wall, inhibiting undesirable microbes and cross-

feeding other beneficial gut microbes, resulting in production of the SCFA butyrate, which fuels intestinal epithelial cells in the large intestine. SCFA may also be absorbed and transported to the peripheral circulation via the portal vein to act as signalling molecules on the liver and peripheral tissues, regulating hormonal and nervous system processes in the host. Thus, SCFA produced commonly by many different probiotic strains and species are an essential contribution to the proliferation of the GIT (Topping & Clifton, 2001). *Lactobacillus* is among the genera capable of converting tryptophan to indolealdehyde, an indole metabolite that has positive impact on neuroendocrine function and immune response. These microorganisms may not necessarily be constant inhabitants of the GIT but colonisation isn't necessary for probiotics to have a functional impact on the structure of the GM beyond their temporary effects (Hungin *et al.*, 2018).

2.4.1.2 Lactic Acid Bacteria Fermentation

The group of LAB has a long and safe history of application and consumption in the production of fermented foods and beverages, thus are very important to the food industry (Holzapfel, 1995). Fermentation is an ancient technology that began as a spontaneous process which occurred due to wild microflora that are naturally present in the environment. The utilisation of LAB by humans has a very long history and the earliest record of human use of LAB can be dated back more than 10,000 years ago as supported by archaeological evidence (Bintsis, 2018). Though likely a spontaneous rather than intentional event, the first fermentation dairying occurred more than 8000 years ago when North African residents consumed natural acidified milk products. Small amounts of previously generated fermentations could be used to inoculate new batches repeatedly and infinitely, as in the production of sourdough bread. Production of fermented foods is a cost effective and reliable preservation method, and additionally is appreciated for unique gastronomic virtues (Leroy & De Vuyst, 2004).

A starter culture can be defined as a microbial preparation of a large number of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its acidification process. The production of lactic acid via thermophilic fermentation of lactose by the starter culture is the foundation of cultured milk products such as yoghurt. LAB are commonly employed in

the food industry, with their main application as starter cultures for fermented dairy products. Starter cultures are added directly to raw materials to initiate the acidification process and achieve a controlled fermentation. The resultant acidifying compounds contribute to sensory attributes such as aroma, flavour, and texture with a characteristic tang. When homofermentative LAB convert sugar (lactose in the case of dairy products) into lactic acid via pyruvate, other metabolites may be produced, lending to the typical flavour of certain fermented foods, such as ethanol (kefir) and acetaldehyde (yoghurt) (Bintsis, 2018). *Lactobacillus delbrueckii* subsp*. bulgaricus* and *Streptococcus thermophilus* are two species with a symbiotic interaction for growth and acidification, making this co-culture ideal for yoghurt manufacturing. The synergy between these two homofermentative species is the basis for successful milk fermentation and gelation. Early in fermentation, *S. thermophilus* leads the acid production and reduces pH to an optimal level for *L. bulgaricus* to grow and start also metabolising lactose into lactic acid. Lacking essential proteolytic activity, *L. bulgaricus* grow slowly in milk and are associated with high acidity and bitterness, thus they are usually combined with non-proteolytic *S. thermophilus* for a more effective fermentation, more rapid acid production, and creation of flavour compounds (Sieuwerts, 2016).

Acid gelation is a fermentation process that requires the associative action of two or more species. At the initial stages, Streptococcus species convert lactose to lactic acid, and as the concentration of lactic acid increases to 1% Lactobacilli start acting at around pH 5. Caseins are coagulating proteins in milk that assemble into micellar structures by the joining of phosphoserine molecules to each other through molecules of amorphous calcium phosphate. The casein micelles are suspended in milk through steric stabilisation forces and they have a negative charge at pH 6.7. As the pH of milk drops from 6.7 to 4.6, calcium phosphate gets solubilised causing the caseins to fall apart from the micelles. Protonation of the caseins takes place as acidification progresses. The isoelectric point is reached at pH 4.6, when the caseins are not charged and fall out of solution. This is the point of acid gelation and it is believed that the casein chains entrap water and form loose networks with each other. The gel formed in this way is called yoghurt (Huppertz *et al.*, 2018).

In the production of yoghurt, specific procedures and growth conditions must be met. First the milk is standardised, or adjusted for the desired content of solids, casein, and fat. Next the milk is homogenised to increase the number of fat particles that contribute to an increased viscosity and in addition reduces whey separation. Some fermented dairy products such as sour cream undergo a second homogenisation which encourages clustering of fat globules that further increases the viscosity of the product. Pasteurisation prevents the growth of any competing microbes and spores and reduces the microbial load of other non-pathogenic bacteria. Also, oxygen level is reduced, creating an improved environment for starter bacteria to grow. Heat processing at or above 90°C denatures whey. The milk is cooled to an optimal temperature for the relevant bacteria culture before the starter is added and the mixture is incubated, usually between 40-45°C for *L. bulgaricus*. Temperature is a critical variable that influences kinetic progress (and therefore acidification and rate of gel formation) during fermentation. Acidification by LAB impacts the gelation of casein and at around pH 5, caseins begin to denature and reassemble in a network of molecules. These casein micelles are the building blocks of casein-based gels that provide the distinctive coagulated texture of yoghurt. This growth phase and acid production continues until a final pH of 4.2-4.5 is achieved and the gel is formed, at which point the cultures start to become inhibited by the low pH and excess of acid and hydrogen peroxide. Too short or long of a fermentation time also results in impairment of consistency and flavour, therefore the yoghurt is usually blast chilled then stored at 5°C to slow down degradation. Gel viscosity and firmness increases in the cooled product due to an increase in the size of casein particles (Lucey, 2004).

2.4.2 Effects of Sweeteners on Starter Cultures

The introduction of sweeteners into a food product can result in significant sensory alterations, particularly in appearance, texture, and flavour; yet there is little known about the influence of sucrose substitutes on the viability of probiotic bacteria. Retention of viability of probiotic cultures during processing is a major challenge in the development of functional foods (Farnworth, 2003). The inclusion of live active cultures into a food matrix has been recognised to impart several technological challenges which may detrimentally affect their growth, viability, and functionality

during production and storage (Lee & Salminen, 2009). Stress factors the bacteria may encounter include temperature, pH and titratable acidity, and exposure to osmotic and oxidative stress in product matrices (Mohammadi & Mortazavian, 2011). Additional key factors are the presence of antimicrobial agents such as hydrogen peroxide, bacteriocins, SCFA, some flavouring agents, salt, sugar, sweeteners, artificial flavouring, colouring agents, and other additives. In order to deliver their health benefits, probiotics must adapt and survice in variable environments (Terpou *et al.*, 2019).

Conjugated linolenic acids (CLA) are a diverse group of positional and geometric isomers of linolenic acid with conjugated double bonds. CLA have received a nutraceutical food status, and they are predominantly found in milk and meat products. LAB produce CLA from linolenic acid, and CLA conversion by LAB can be influenced by bacterial strain, incubation time and cell age. Lipolytic activity of starter cultures has been shown to be influenced by the addition or modification in ingredients in yoghurt and can result in modified fatty acid profiles. In the presence of sucrose, the inhibition of fatty acid production in starter culture was observed. In a study which looked at the effects of the addition of sucrose, lactose, and fructose on CLA level in skim milk medium after incubation varied with lactic cultures, all three sugars strongly inhibited the level of CLA produced by *L. delbrueckii subsp. bulgaricus* and *L. acidophilus*. Sucrose and fructose showed an inhibition on CLA produced by *L. delbrueckii* subsp*. lactis and S. thermophilus*, yet conversely, fructose promoted an increase in CLA level of 22% with *Lactococcus lactis* subsp*. cremoris*. This demonstrates the variable specificity of probiotic strains and how compatibility with different food ingredients plays a major role in their survival (Lin, 2000).

NNS were evaluated for their effect on the viability of probiotic cultures in yoghurts. The sweetener ace-K and aspartame were observed to not be inhibitory towards lactic acid starter at the concentration normally used in dairy drinks, however at a higher concentration, aspartame was inhibitory towards *S. thermophilus* and two strains of *L. lactis* (Vinderola *et al.*, 2002). A different study which determined probiotic survival in yoghurts showed that NNS in place of sucrose showed no negative impact on probiotic viability (Esmerino *et al.*, 2013). Research has indicated that some fruits have the potential to perform as substrates to improve probiotic viability, while

other fruits have demonstrated inhibitory effects (Vinderola *et al.*, 2002). Yoghurts with stir-in fruits have shown improved survival of L. acidophilus, possibly due to a reduction in *L. bulgaricus.* However, some fruit juices or pulps may adversely affect probiotic survival, depending on bacteria strain, due to higher acidity or antimicrobial components in the fruits (Shori, 2016). In a study that added monk fruit extract to yoghurt, there was a significant enhancement of *L. casei* viability, along with antibacterial activity against *E.coli, Salmonella typhimurium*, and *Listeria monocytogenes,* compared to a control yoghurt with no sweetener added. It is believed that this antimicrobial action of monk fruit extract is an enhancement to the antibacterial peptides released during the hydrolysis of milk protein by the yoghurt cultures (Abdel-Hamid *et al.*, 2020).

2.5 Prebiotic Fibres and Non-Digestible Carbohydrates

Dietary carbohydrates are generally classified based on their digestibility by the small intestine: “easily digestible” or “non-digestible” (*Figure 7*). Digestible carbohydrates are either absorbable without digestion or are enzymatically degraded in the small intestine, releasing glucose into the bloodstream and stimulating an insulin response. Included in this group are starches and sugars, such as glucose, fructose, sucrose, and lactose. Conversely, non-digestible carbohydrates such as fibre and resistant starch escape degradation by enzymes in the small intestine and instead travel to the large intestine where they undergo fermentation by resident microorganisms (Singh *et al.*, 2017). Dietary fibre is nondigestible carbohydrates found in plants, including plant nonstarch polysaccharides, such as cellulose, pectin, gums, hemicelluloses, β-glucans, algae derivatives, lactulose, inulins, fructooligosaccharides, galactooligosaccharides, xylooligosaccharides, and isomaltooligosaccharides. Functional fibres are defined to be isolated, extracted, or synthetic nondigestible carbohydrates that have proven physiological benefits in humans. They include isolated, nondigestible fibre from plants (e.g., resistant starch, pectin, and gums), animal derived (e.g., chitin and chitosan), or commercially produced carbohydrates (e.g., resistant starch, polydextrose, inulin, and indigestible dextrins) (Brownawell *et*

al., 2012).

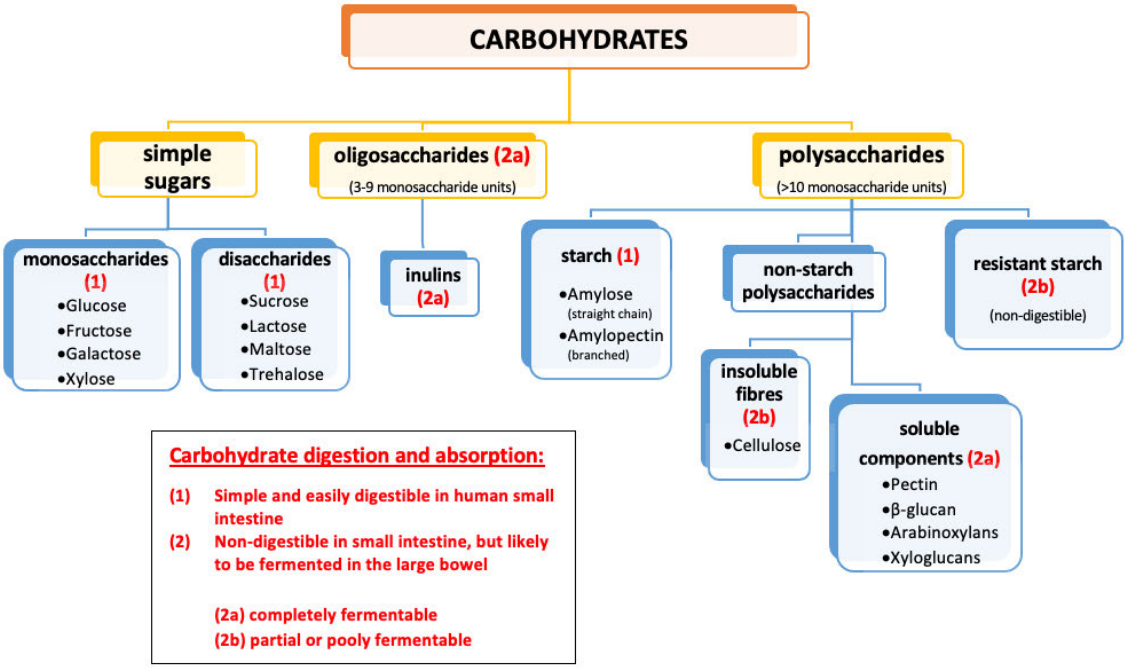

Figure 7
*Dietary Carbohydrates and Their Potential for Digestion and Absorption in the Human Body (*Adapted from (Barba, 2019).

2.5.1 Prebiotics

Short chain carbohydrates that are non-digestible but fermentable in the colon are collectively known as prebiotics. In order for a food ingredient to be considered a prebiotic, it must be i) resistant to gastric acidity, hydrolysis by enzymes, and gastrointestinal absorption; ii) fermentable by intestinal microflora; and iii) selectively stimulate the growth and/or activity of intestinal bacteria associated with health and wellbeing. Certain indigenous GI bacteria selectively metabolise a prebiotic, allowing specific changes in the composition and/or activity of gut microflora thus conferring benefit(s) to the host (Gibson *et al.*, 2017). The benefits of prebiotic compounds include an increased absorption of minerals such as calcium, production of vitamins, and inhibition of harmful bacteria in the GIT; in addition to increased satiety leading to a decrease in calorie intake which can support weight control (Gomes Da Silva *et al.*, 2017). Prebiotic and dietary fibre are terms both used to describe food components that are not digested in the GIT, however, while all prebiotics are fibre, not all fibre is

prebiotic. Since this is a selective stimulation, modifications to the gut microbiota occur at the level of individual strains, and dietary fibre is used by the majority of colonic microorganisms. Based on the number of monomers bound together, prebiotics may be classified as: disaccharides, oligosaccharides (3-9 monosaccharide units), and polysaccharides (10 or more monomers) (Markowiak & Śliżewska, 2017). The most important prebiotics are inulin and oligofructose, which are soluble and fermentable fibres. They are named fructans and cannot be digested by α-amylase or other hydrolases in the upper section of the intestinal tract (Gibson *et al.*, 2004).

Prebiotics can be found in fruits, vegetables, cereals, and other edible plants. Some prebiotics (i.e., inulin) occur naturally in several foods such as leeks, asparagus, chicory, Jerusalem artichokes, garlic, onion, wheat, oats, and soybeans. Since prebiotics are naturally contained in fruits and vegetables, they offer a direct approach to act as a carbohydrate source for probiotic bacteria and manage the microflora through diet. In addition to the benefits they bring, certain prebiotic sources also provide sweetening power while contributing low caloric value, making them potential alternatives to artificial sweetening substances. Foods may be fortified with prebiotic ingredients, such as inulin, FOS, and galactooligosaccharides. These β-linked oligosaccharides are polymers of fructose linked by a β (2→1) glycosidic bond. As the β (2→1) bond is resistant to cleavage in the small intestine, these pass into the colon where they are metabolised by the gut bacteria and ultimately affect gastrointestinal health (Barba, 2019).

2.5.2 Prebiotic Impact on Probiotic Bacteria

Many studies suggest that a diet rich in non-digestible carbohydrates consistently target *Lactobacillus* and *Bifidobacterium* in the gut, and these are the most popular target genera for prebiotics (Slavin, 2013). When prebiotics and probiotics are combined in a food product, the combination is called a synbiotic (De Vrese & Schrezenmeir, 2008). Prebiotics can also modulate the colonic flora by inhibiting pathogens such as *Clostridium* and *Bacteroides* (Holzapfel & Schillinger, 2002). Besides the established positive impact on microflora, other indirect but beneficial effects of prebiotics include production of SCFA (lactic, acetic, propionic, and

butyric acid) which may play a role in primary prevention of colorectal cancer, immunomodulation, influence on sugar digestion and absorption, and glucose metabolism. As intestinal microbiota are stimulated by prebiotics, fermentation activity is determined and concurrently this influences SCFA levels. Because of fermentation in the large intestine, the ingestion of higher quantities of prebiotics may lead to GI intolerance symptoms including flatulence, abdominal disorders, and diarrhoea, due to osmotic potential and/or excessive fermentation (De Vrese & Schrezenmeir, 2008). Some known prebiotic fibres are associated with impaired gastrointestinal tolerance, while other prebiotics exhibit high gastrointestinal tolerability (Slavin, 2013). One study established that the prebiotic source yacon is well tolerated and does not cause gastrointestinal problems when ingested at a dose of 0.14g/kg daily (Ojansivu *et al.*, 2011). In addition to the dietary dosage, prebiotic effectiveness and stability of inulin-type fructans depends on the DP, which can be affected by processing conditions such as temperature or pH, with FOS hydrolysis increasing with temperature, and decreasing with an increase in pH (Gomes Da Silva *et al.*, 2017).

2.6 The Gut Microbiome

The gut microbiome (GM) is a complex community of trillions of microorganisms that reside in the intestine. The microbes themselves are an assemblage known as the microbiota. In the past decade, gut microorganisms have been increasingly recognised as having a wide variety of fundamental roles and functions which contribute to human health. In addition to playing a central part in modulating immunity and protecting against pathogens, the microbiota has been associated with numerous chronic conditions including inflammatory bowel disease (IBD), T2DM, and diet induced obesity (Ley *et al.*, 2005). Facultative aerobes are the earliest colonising species in the infant gut, with an eventual shift to an anaerobic environment led by facultative anaerobes such as lactobacilli, enterococci, and enterobacteria. A commensal enteric microbiota is rapidly acquired creating the complex ecosystem characterised by an abundance of diverse inhabitants including protozoa, yeast, viruses, and hundreds of species of bacteria. Amongst the bacteria, Firmicutes and

Bacteroidetes predominate, with these two phyla representing more than 90% of the gut microbiota (Donaldson *et al.*, 2015). The structure and composition of the human gut microbiome has been implicated in most aspects of health and disease, thus modification of microbiota has progressively gained more attention as a treatment for several diseases in humans and animals. Studies have shown that the microbiota in obese humans is overall a less diverse composition with a higher abundance of Firmicutes, hence displaying a higher Firmicutes: Bacteroidetes ratio (Singh *et al.*, 2017). Experiments that transplanted microbes from obese humans into the gut of germ-free mice (raised in the absence of any microbial life) exhibited a significant increase in body fat percentage, showing that the obese phenotype is transmissible (Turnbaugh *et al.*, 2006). It remains unclear whether an alteration of microbiota leads to obesity, or if weight gain leads to changes in the GM.

It is now understood that the microbiota has the potential to impact human metabolism and extract nutrition and energy from foods by synthesising essential vitamins and amino acids, and by generating SCFA (Thursby & Juge, 2017). Many LAB and other Firmicutes species are known to be major producers of SCFA, such as butyrate, propionate, and acetate, which are thought to strengthen the mucosal barrier and act as key metabolic mediators (Zhang *et al.*, 2018). Through the production of metabolic end products, LAB decrease luminal colonic pH to a level that pathogens can not withstand. Many LAB are able to excrete natural antibiotics which have a broad spectrum of activity. Butyrate has a beneficial effect on glucose and energy homeostasis, propionate regulates gluconeogenesis and satiety signalling, and acetate may play a role in central appetite regulation (Valdes *et al.*, 2018). Consistent with this, observational studies have correlated SCFA with improved postprandial glycaemic response, reduced insulin resistance, lower diet-induced obesity and lower incidence of T2DM (Ley *et al.*, 2006). The significance of microbial variability is increasingly being recognised, with low microbial diversity directly corresponding to a state of disease, known as dysbiosis. Through studies on twins, it has been established that although there is a heritable component to gut microbiota composition, the larger determinants are environmental factors such as diet and drugs, and anthropometrics (Rothschild *et al.*, 2018). The diversity of gut microbiota has also been observed based on geographic regions and the diets of different cultures were associated with variable

microbiota profiles. Another factor which was correlated with microbiota composition is the socio-economical differences among individuals in the same geographic provenance. Dietary intervention is, however, one of the critical contributing factors to the microbiome composition and diversity (Senghor *et al.*, 2018).

2.6.1 Effect of Diet on Microbiota

Specific types of foods and dietary patterns have been evidenced to induce dynamic shifts in existing host bacterial genera, with impact that can be produced in as little as 24 hours. Certain diets are correlated with changes in GM abundance and composition, such as the increase in bile-tolerant microorganisms seen with an animal-based diet, and the increase in Firmicutes resulting from a plant-based diet. In patients with IBS, the low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet that is commonly prescribed to reduce symptoms also reduces overall bacterial count, including the beneficial SCFA producing microbes (David *et al.*, 2014).

The long-term weight gain that is evidenced by a mouse study correlates with low microbiota diversity and is also associated with a low intake of dietary fibre (Wang *et al.*, 2017). Because of the well-known causality of the intestinal microbiota on human health, some important and necessary research is looking at the effects certain food additives have on microbes (Sonnenburg & Bäckhed, 2016). Common additives such as emulsifiers have been shown to affect mouse gut microbiota, promoting colitis and metabolic syndrome (Suez *et al.*, 2014).

2.6.2 Effect of Sweeteners on Microbiota

2.6.2.1 In Vitro and Animal Studies

In addition to the general health concerns about NNS, there is a growing body of evidence that sugar substitutes have a negative effect on gut microbiome composition, and some sweeteners have been reported to induce effects on select bacterial genera (Table 2). NNS stimulate the development of glucose intolerance through compositional and functional changes in gut microbiota, which in turn leads to sensitivity of the host to metabolic disorders. One study's findings demonstrate that aspartame, sucralose, and saccharin disrupt the balance and diversity of gut

microbiota, mediating a strongly altered glycaemic response which leads to glucose intolerance (Suez *et al.*, 2014). The same authors furthered the proposed association of NNS and altered gut microbiota by performing fecal transplantation in germ free mice. When the mice received transplantation with microbes from a saccharin-consuming donor, metabolic derangements ensued. The induction of hyperglycaemic response in the mice confirms the existence of glucose response mediated by the gut microbiome as a result of NNS (Suez *et al.*, 2015). However, a different author notes that there is a limitation presented by the fact that that the dose of saccharin used in the experiment was several-fold higher than typical human consumption, therefore it is uncertain whether the results could be reproduced in humans (Imamura *et al.*, 2015).

NNS were shown to have a functional impact on gut microbiota via inflammatory processes that promote the development of chronic disease caused by microbiota dysbiosis. The inflammatory metabolites that are regulated by gut bacteria were shown to lead to reduced metabolic activity in mice (Bian *et al.*, 2017c). Saccharin consumption induced the development of these metabolites, altering the dynamics of gut microbiome development, and causing a pro-inflammatory effect in mouse liver (Bian *et al.*, 2017b).

Ace-K was shown to have a strong direct bacteriostatic effect on *E. coli* in the GM of mice. The Firmicutes phylum became significantly more abundant, and Bacteriodetes showed a trend towards less abundance. The research showed the same bacteriostatic results with administration of saccharin and sucralose (Wang *et al.*, 2018). A study of rats that were fed aspartame resulted in a reduction in energy intake though they displayed an elevation in fasting glucose levels and impaired insulin response. Aspartame induced distinctive changes in the GM composition with an increase in propionate producing bacteria, an effect that was amplified in the obese state. Since propionate is a highly gluconeogenic substrate, this is a potential explanation of aspartame's negative effect on insulin tolerance (Palmnäs *et al.*, 2014).

Rats that were given a 12-week treatment of sucralose at dosage within the range of FDA acceptable daily intake had lower viable counts of aerobic and anaerobic faecal bacteria. A significant increase to intestinal pH was also seen, which is noteworthy since it can modify absorption of nutrients and bioavailability of drugs in

the GIT. Following a 12-week recovery period, total anaerobes and bifidobacteria remained depressed (Abou-Donia *et al.*, 2008). A different study administered sucralose in an amount equivalent to the FDA approved acceptable intake, and there was a disruption in faecal metabolites that are involved in regulation of inflammation, in addition to an increase in the expression of bacterial pro-inflammatory genes in the altered GM (Bian *et al.*, 2017b).

***Table 2:** Effects of Select NNS on Gut Microbiota in Animal Studies*

Sweetener	Model	Microbiota	Outcomes	Reference
Acesulfame K	Rats	*E.Coli*	Bacteriostatic effect	Wang *et al.*, 2018
	Mice (Male)	*Bacteroides, Anaerostipes and Sutterella*	Increase in populations	Bian *et al.*, 2017a
	Mice (Female)	*Lactobacillus, Clostridium and Ruminococcacaeae*	Decrease in abundance	
Aspartame	Rats	*Enterobacteriaceae and Clostridium leptum*	Increased numbers	Palmnäs *et al.*, 2014
	Rats	*Enterococcaceae*, *Enterococcus*, and *Parasutterella*	Reduced abundance	Nettleton *et al.*, 2020
		Clostridium cluster IV	Increased abundance	
Saccharin	Mice	*Bacteroides and Clostridiales*	Increased abundance	Suez *et al.*, 2014
		Firmicutes	Reduced abundance	
	Rats	*Lactobacillus*	Growth inhibition	Naim *et al.*, 1985
Sucralose	Rats	*E.Coli* HB101	Reduction in number of colonies and colony size	Wang *et al.*, 2018
	Mice	*Ruminococcus, Streptococcus*	Decrease in numbers	Bian *et al.*, 2017b
	Rats	*Bifidobacteria, Lactobacilli, and Bacteroides*	Reduction in total numbers	Abou-Donia *et al.*, 2008

2.6.2.2 Human Studies

Most human microbiome studies that use faecal specimens for microbial analysis are limited in relevance since they are poorly representative of microbiota of the upper proximal intestine. A human trial which looked at the short-term intake of ace-k and aspartame reported no differences in median bacterial abundance across consumers and non-consumers, however it was indicated that there were significant differences in overall bacterial diversity, reducing in diversity from 24 to 7 phyla

(Frankenfeld *et al.,* 2015). It is suggested that future researches may improve methods for sample collection by utilising capsule endoscopy (Hollister *et al.*, 2014). The first work to characterise the human gut enterotype demonstrated the impact of long-term diet on the gut microbiome using a combination of diet recall and faecal analysis (Wu *et al.*, 2011). The impact of short-term diet on GM composition was also demonstrated using a similar research structure (David *et al.*, 2014).

Though many studies evidence the ill effects of aspartame on human health, human trials on the effects on GM composition are lacking. There are no studies on the effect of aspartame on the GM specifically, however it is completely hydrolysed in the small intestine and does not reach the colon (Magnuson *et al.,* 2016). The faecal transplant model has given some informative results for studying the human GM. Mice recipients of microbiomes from saccharin consuming human donors resulted in an altered microbiome, compared to the control mice. Saccharin was shown to increase the number of bacteria that are associated with obesity, and functionally alter the metabolic pathways that are linked to glucose tolerance and obesity (Suez *et al.*, 2015). In order to establish some biological plausibility, research has quantified the effects of NNS on SCFA production using in vitro faecal culture profiling, which can be extrapolated as biomarkers of human microbiome health (Laudisi *et al.*, 2019). By analysing metabolite formation, conclusions may be drawn regarding microbiota interaction with NNS. Indicative of a metabolomic response, Ace-k was shown to increase levels of butyrate and pyruvate, and sucralose altered the ratio of butyric and propionic acids, compared to a control. Butyric acid is a known to work against obesity and insulin resistance. Lowered concentrations of these SCFA have been correlated with IBS. SCFA levels were measured in human colon simulators to determine the impact of NNS on metabolic activity of microbiota. A decrease in the number of genomes of the Firmicutes was caused by saccharin and sucralose, having a direct correlation with reduced levels of SCFA, and linking NNS to disturbances in the normal microbial pattern. Also negatively affecting the microbiota balance, an increase in Gram-negative bacteria was observed (Vamanu *et al.*, 2019).

Changes to microbial and metabolomic patterns can cause physiological dysfunctions that trigger chronic disease. Though it is difficult to translate the findings with animal models to gut microbiota interactions in humans, a study was able to

show similarity to human pathology of Chron's disease through a mouse model. The research evidenced dysbiotic changes in the gut microbial ecosystem, and chronic ileal inflammation due to sucralose consumption, which can increase susceptibility to colitis (Wang *et al.*, 2019a). NNS induce changes in the gastro-intestinal environment and thus of microbiota; which can trigger glucose intolerance (Palmnäs *et al.*, 2014). Early studies have indicated that artificial sweeteners maintain peak insulin concentrations and plasma glucose without affecting gut microbiota. More recently, animal and human studies presented specific changes in microbiota as a result of alterations in the metabolic pathways which are related to glucose tolerance and dysbiosis in human subjects, especially with the ingestion of saccharin (Suez *et al.,* 2014). Saccharin, ace-k and sucralose have been found in the breastmilk of nursing mothers. Immature clearance mechanisms in infants means lower filtration rates that could equate to intake levels exceeding the ADI which is established for adults. Because healthy gut microbiota plays an important role in protection against chronic disease during early infancy, NNS ingestion by infants is particularly concerning (Sylvetsky, 2018).

2.6.3 Effect of Probiotics and Prebiotics on the Microbiome

Reduction of gastrointestinal infections and disorders can be achieved through the intake of probiotics. Increases in *Lactobacillus* and *Bifidobacterium* intake have been described as an effective defence against gut dysbiosis. These effects may be due to diverse modes of action such as direct antimicrobial activity through production of bacteriocins, or competition for binding sites or stimulation of colonocyte function. Changes in GM function and composition are marked features of obesity, and probiotic bacteria can create a balance between pathogens and microbiota. Strains such as *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus,* and *Enterococcus* are used as a therapeutic strategy to treat and prevent obesity via modulation of microbiota. Three main mechanisms of obesity treatment by probiotics have been defined as follows: 1) antagonistic effects on pathogenic microorganism growth and competitive adherence to intestinal mucose and epithelium (antimicrobial activity), 2) increased intestinal mucus layer production and reduced intestinal permeability (barrier function), and 3) modulation of the gastrointestinal immune system (immunomodulation) (Cerdó *et al.*, 2019).

It has been confirmed by animal studies and human clinical trials that the intake of prebiotics can modify both intestinal microbiota composition and abundance. FOS and GOS are known substrates to stimulate the growth of *Lactobacillus* and *Bifidobacterium* in the gut. As an indirect effect resulting from the growth of these beneficial species, prebiotic yacon root interacts with the immune system, influencing the production of cytokines and creating an immunomodulatory effect in both humans and animals (Gibson & Roberfroid, 1995). In a study which administered yacon flour to mice, an increase in bifidobacteria and lactobacilli counts was observed, and enterobacteria population was significantly diminished in the intestinal microbiota, compared to the non-yacon consuming control group (Bibas Bonet *et al.*, 2010). Other prebiotics have variable effects on the microbiota due to different degrees of polymerisation. The prebiotic metabolism end products, organic acids and SCFA, are known influencers of the intestinal microenvironment, reducing colonic pH, increasing beneficial microbe inhabitants and blocking pathogens. Populations of some intestinal bacteria decreased after intake of prebiotics, possibly due to competitive inhibition from other intestine colonising species that preferentially ferment prebiotics (Wang *et al.*, 2020).

Synbiotics combine probiotic and prebiotic components with the aim of increasing the abundance of beneficial microbes and correcting the disruption to gut microbiota which may be observed due to obesity or an imbalanced diet. A clinical trial evaluated body composition and biomarkers of obesity as related to the intake of a combination of probiotic bacteria (*L. acidophilus, B. bifidum*) and prebiotic GOS. Synbiotic supplementation increased the proportion of species known to be correlated with lower body mass and BMI, such as *Lactobacillus* and *Bifidobacterium*. Other beneficial effects include a decrease in fasting blood glucose level and a positive effect on other metabolic parameters (Markowiak & Śliżewska, 2017). Furthermore, significant alterations in gut microbiota composition were observed. The synbiotic intervention created an increase in the butyrate-producing genus *Ruminococcus*, thus benefitting colonocytes and protecting against toxic metabolites that are linked to cancer (Sergeev *et al.*, 2020).

3 Materials and Methods

3.1.1 Materials

The starter used in this study was a commercial food-grade freeze dried co-culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (NPSelection, United Kingdom). Starter cultures were stored at 5°C until used. Three alternative sweeteners were selected, lucuma fruit and yacon root, both in the form of a ground whole food powder/flour (Buy Whole Foods Online, United Kingdom), and monk fruit containing 25.0% Mogroside V, a high-intensity sweetening powder extracted from the luo han guo fruit (Buddha Fruit, United Kingdom). The sweeteners were used as they were without any further purification. UHT skimmed milk and table sugar were purchased from a local supermarket (Tesco, United Kingdom). All other chemicals used were of analytical grade and purchased from Sigma-Aldrich (Dorset, United Kingdom).

3.1.2 Sweetener Concentration

A preliminary tasting was conducted in order to determine the sweetening power and define the concentration of sweetener to be used in the experiments. A standard 1.0%wt sucrose solution was prepared, along with two concentrations of the sweeteners (1.0% and 5.0%wt). Two individuals evaluated the relative sweetness of yacon, lucuma, and monk fruit, against the sucrose solution, drinking water and pausing for 10 minutes in between. The lucuma and yacon samples had barely any perceived sweetness at 1.0%wt and the ideal concentration was determined to be 5.0%wt. The solubility of lucuma and yacon decreased dramatically when the concentration was above 5.0%wt.

3.1.3 Innoculum Preparation

To prepare the mother culture, starter culture was inoculated into the sterile UHT skimmed milk in the amount of 1g per 100mL then placed in an incubator (Thermo Scientific Herathern Incubator IGS60) for 18 hours at 45°C. This prepared mother culture was then placed into storage and held at -25°C.

3.2 Acidification Profiling

The sweeteners were tested for the effect of several concentrations on pH during fermentation. Acidification profiling of cultured milk in the presence of the selected sweeteners was performed according to Oliveira *et al.* with some modifications (Oliveira *et al.*, 2009). Three different sweeteners (lucuma, yacon, and monk fruit) were added at 5.0%wt into sterile skimmed milk. 50 mL aliquots were prepared containing 50% (25 mL) of pure skimmed milk with sweetener and 50% (25 mL) of pre-activated mother culture made with 1.0% starter. Additionally prepared was a control series of 6 tubes of only pure milk and pre-culture, without any added sweetener. All tests were run in triplicate. Tubes were mixed using a vortex (Velp Scientific 2x3 Advanced Vortex Mixer) and placed into 45°C incubator for fermentation. After taking the initial readings for the zero-hour samples, pH was monitored at one-hour intervals using a digital glass electrode pH-meter (Hanna Instruments pH300, United States). Readings were taken every hour up until 5 hours when fermentation was complete (pH reached 4.5) and the results were plotted for acidification profiling. Aliquots from each time interval were stored at -25°C until used for the methylene blue test.

3.2.1 Kinetic Parameters

The acidification profiling data was used to calculate the maximum acidification rate, or v_{max}, as the time variation of pH units, expressed as time in hours (h^{-1}). The following kinetic parameters were also calculated during the incubation period: t_{max}, or the time required to reach v_{max}; $t_{pH5.0}$, or the time at which the fermentation reached pH 5.0; $t_{pH4.5}$ (t_f), or the time at which the fermentation reached pH 4.5 indicating the completion of fermentation (Oliveira *et al.*, 2009). The reported values are averages from three replications.

3.2.2 Methylene Blue Test

Lactobacillus delbrueckii subsp. *bulgaricus* are characterised as Gram-positive, facultatively anaerobic, non-motile and non-spore-forming, rod-shaped bacterium, with a cell size range of 0.5-0.8 x 2.0-9.0 µm (Teixeira, 2014). *Streptococcus thermophilus* are a Gram-positive bacterium with non-motile spherical to ovoid cells of

0.7 - 0.9 µm in diameter (Harnett *et al.*, 2020). Methylene blue staining was employed to determine cell viability based on the uptake of the dye by dead or injured cells. Viability is defined as the relative proportions of colourless (live) cells in a whole population (Evans, 2006). 100 uL of 10^{-1} culture dilution was wet mounted onto a glass slide with pipette followed by one drop of 0.01% methylene blue dye, allowing excess to drain off the slide onto a paper towel. Living cells are able to reject the dye and dead cells absorb it and are stained blue due to the uptake of dye. The slides were viewed under the compound microscope (Leica Microsystems Model DMR, Illinois, United States), and pictures were taken under 400x magnification (IC Capture, Imaging Source, North Carolina, United States). Images displayed the distinction between live and dead cells, and cells which appear blue were considered to be nonviable. Cell viability was calculated as a percentage of live cells to total number of cells. A square measuring 150x150 pixels was placed over the slide images, with 5 locations selected at random, to complete a manual cell count.

3.2.3 Statistical Analyses

The effect of the selected natural alternative sweeteners on the acidification of skimmed milk during fermentation and cell viability (methylene blue test) was investigated with a one-factor-at-a-time experimental design. A descriptive analysis was conducted to ensure all data collected was normally distributed (Shapiro-Wilk test) and had equal variances (Levene test). One-way analysis of variance (ANOVA) and Tukey's Post Hoc tests were performed using IBM SPSS Statistics 22 to examine if there was a significant difference among the samples. The values were considered significantly different when $p < 0.05$. Values presented are the means of experiments done in triplicate ($n = 3$).

4 Results and Discussion

4.1 Acidification Profiling

Figure 8 shows the change in pH of the cultured milk samples with 5.0%wt sweetener, incubated at 45°C for 5 hours. A control without any sweetener was included for comparison purposes. The general trend of pH change was a slower more gradual start as this was the lag phase of bacterial growth, with the pH dropping more rapidly as the LAB produced more lactic acid at a later stage as it was fermenting and the bacterial growth rate entered the exponential, or log phase. This growth curve was a straighter line with less of a slope when compared to the sigmoid or bell shaped curve that is demonstrated with a comparative study of an acidification profile of in the presence of inulin, which exerts a stronger prebiotic effect than our sweeteners (Oliveira *et al.*, 2009). This study utilised the same probiotic co-culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) as in this study, so it is possible that the differences in the acidification profile are due to the presence of different additives in the milk which alter the growth characteristics and dynamic of the probiotic co-culture.

All samples had a similar pH at the beginning of fermentation but pH dropped faster for the control than all three sweeteners from time 1 hour and towards the end of fermentation. The fermentation of the control samples (represented by pH 4.5) completed at mean of 4.61 hours. Among the sweetener samples, yacon exhibited the slowest decrease in pH, while lucuma dropped the fastest. After 5 hours into the fermentation, the pH of the lucuma sample has dropped by 18.76%, compared to pH change of yacon which declined by 15.54%. The results of the acidification tests suggest that there was a possible inhibition of the starter cultures in the presence of lucuma, yacon, and monk fruit, compared to the cultured milk without added sweetener. While it was expected that there would be an accelerated effect in fermentation, it took slightly longer to complete (represented by pH value 4.5) in samples with all three of the sweeteners.

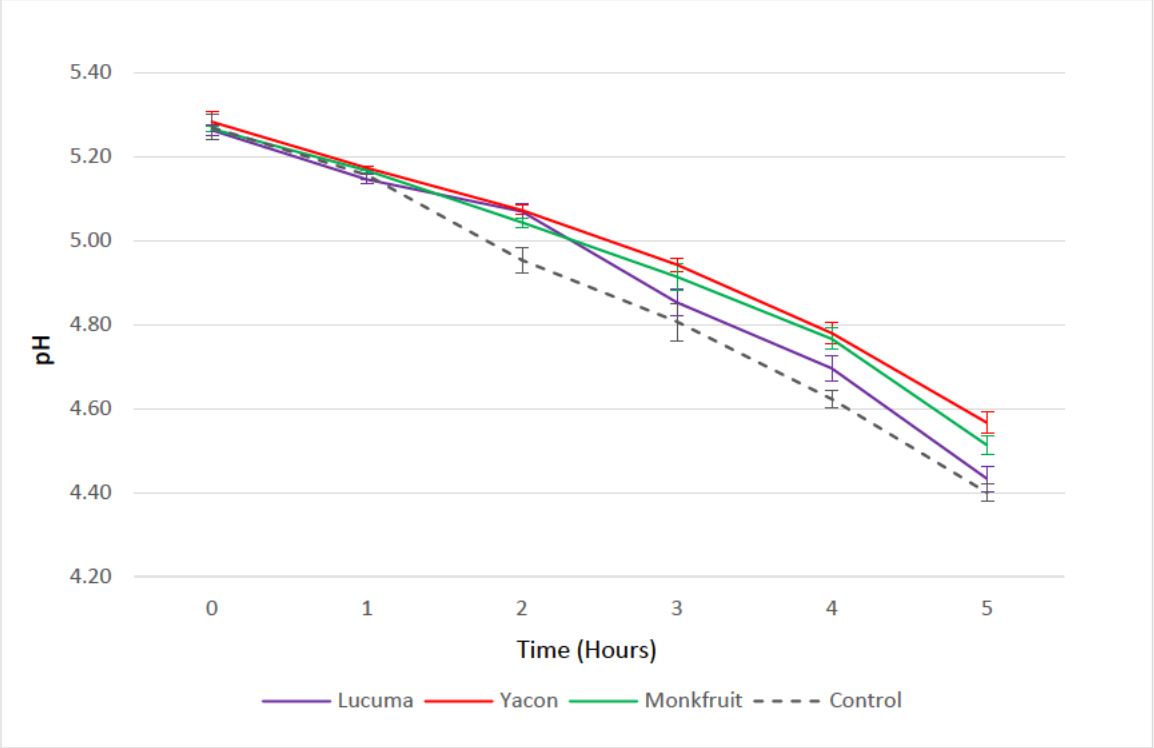

Figure 8
Acidification of Skimmed Milk with 1% Culture in the Presence Of Lucuma, Yacon, and Monk fruit.

4.1.1 Fermentation Kinetics

The acidification profile graphs were used to determine kinetic parameters and the values are reported in *Table 3*. The maximum acidification rate, or v_{max}, was calculated in addition to t_{max}, or the time required to reach v_{max}; $t_{pH5.0}$, or the time at which the fermentation reached pH 5.0; $t_{pH4.5}$, or the time at which the fermentation reached pH 4.5 or completion. It was thought that the cultured milk supplemented with sweeteners would improve acidification rate, but all of the sweeteners actually resulted in a slower fermentation. Lucuma had the highest v_{max}, however values were not statistically significant ($p > 0.05$) between the different sweeteners and the control. The t_{max} values ranged between 2.52 and 4.55 hours, and were not statistically significant ($p < 0.05$). Again, these results confirm that the sweeteners were a hindrance to fermentation rate. Both $t_{pH5.0}$ and the time to complete fermentation were shortest for the control sample, and statistical significance ($p < 0.05$) was observed between all treatments for $t_{pH5.0}$ and $t_{pH4.5}$. The Post Hoc results

show that there was a significant difference ($p < 0.05$) in $t_{pH5.0}$ and t_f values between the lucuma and yacon samples, but not with the monk fruit sample ($p > 0.05$) (*Table 3*).

Table 3

Kinetic Parameters of Acidification - Milk Ferments with Lucuma, Yacon, and Monk fruit

Culture	$v_{max\,(A)}$ (h^{-1})	$t_{max\,(B)}$	$t_{pH5.0\,(C)}$	$t_{f\,(D)}$
Lucuma	0.28 ± 0.02^{a}	4.47 ± 0.03^{a}	$2.28 \pm 0.05^{a,c}$	$4.75 \pm 0.09^{a,d}$
Yacon	0.21 ± 0.02^{a}	4.21 ± 0.56^{a}	$2.55 \pm 0.12^{b,c}$	$5.32 \pm 0.14^{b,c}$
Monk fruit	0.26 ± 0.03^{a}	4.49 ± 0.05^{a}	2.32 ± 0.13^{c}	5.05 ± 0.13^{c}
Control $_{(E)}$	0.24 ± 0.05^{a}	3.81 ± 1.12^{a}	1.73 ± 0.10^{d}	4.61 ± 0.04^{d}

A Maximum rate of acidification
B Time to reach v_{max}
C Time to reach pH 5.0
D Time to complete the fermentation (pH 4.5)
E Control is cultured skim milk with no added sweetener

Different superscripted letters in the same column indicate statistically significant difference among the values of the same parameter ($p < 0.05$).

± Standard deviations with respect to the mean values of triplicate runs.

Some literature has shown that prebiotics have an accelerating effect on LAB, with binary co-cultures reducing fermentation time by about 10%, thus confirming its prebiotic effect (De Souza Oliveira *et al.*, 2009). It was hypothesised for this project that the addition of sweeteners to cultures, especially yacon because of its FOS content, would exhibit a prebiotic effect on the microbes; however this was not observed and there are several possible explanations. Yacon is known to contain prebiotic components, however both the yacon and lucuma used in these experiments are unrefined commercial products, and fibre and FOS content could vary considerably based on factors such as cultivar variety, harvest time and location, and post harvest storage conditions (Cao *et al.*, 2018). It was thought that the sweeteners would improve the acidification rate, but the results suggest that the microbes were unable to utilise the sugars as fermentation substrates; probably because the sugars were entrapped within the fibre matrix of the sweeteners, making them unavailable for prebiotic activity.

Food additives have been demonstrated to significantly influence the growth and cell viability of lactic acid starter and probiotic cultures used in fermented

products, representing the highly specific nature of strains and the variation in their complex nutrition requirements. LAB can be exposed to osmotic stress with the addition of salt or sugar in a food product, and sweeteners have been shown to display an influence on the growth of strains of *Lactobacillus* and *Streptococcus* (Vinderola *et al.*, 2002). It is possible that the sweeteners used in this study presented an impact such as this.

Polyphenols or other bioactive compounds that exist in the sweeteners could either impair or enhance LAB (Terpou *et al.*, 2019). The natural fibres, pigments, alkaloids, bioactive peptides and phenolic acids contained in Andean crops such as yacon and lucuma have been shown to have antibacterial properties and this may explain inhibitory activity (Glorio *et al.*, 2008). Antibacterial bioactivity has been identified in monk fruit, confirming the possibility that it may have hindered microbial growth in this experiment (Abdel-Hamid *et al.*, 2020).

4.2 Percent Viability Calculation

Bacterial viability refers to the ability of a cell to grow and subsequently generate a colony of cells under defined environmental conditions, and this is considered essential criterion for the functionality of probiotics in terms of health-promoting properties (Terpou *et al.*, 2019). *Figure 9* shows the representative light microscope images of the cultured milk samples after 5 hours of fermentation. Methylene blue staining was employed to cultured milk samples. Under magnification, clusters of dead or injured cells that have taken up the blue dye were visible along with the live cells which were clear or transparent. The microscopic images show that there were more dead or injured cells in the cultured milk samples with sweeteners compared to the control. This observation explains the slower acidification rate in the samples with sweeteners. The sweeteners appear to possess some antibacterial activity towards the co-culture although further investigation is required to verify this.

A rough estimation was made of the viable to dead cell ratio from the microscopic images and results are presented in *Table 4*. All samples have a similar percentage viability, ranging from 75% to 85%. Statistical analysis results show that there is no significant difference ($p > 0.05$) in cell viability percentage between all

treatment groups, although the control does have a slightly higher viability percentage compared to the samples with sweeteners. It should be noted that the results are a rough estimate of call viability based on manual cell count on microscope images. A more accurate method such as flow cytometry would be needed for a more definite result. Since LAB strains may exhibit different responses to stress factors, this can affect cell membrane integrity and consequently affect viable cell counts. It is worth mentioning that some studies have evidenced that dead cells can also provide beneficial outcomes for microbiota (Terpou *et al.*, 2019).

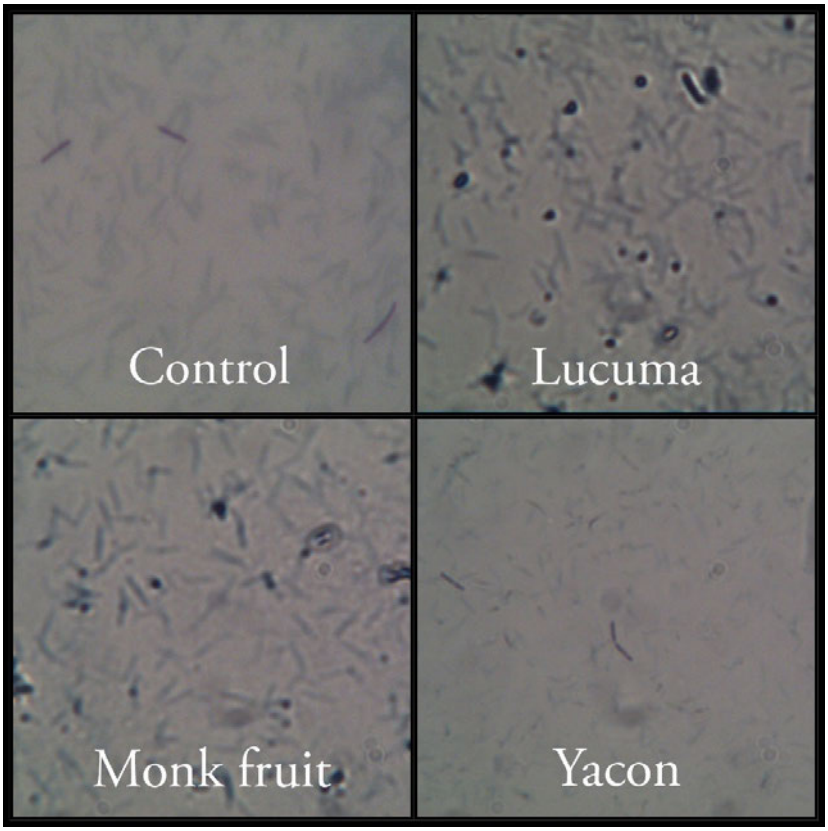

Figure 9
Methylene Blue Stain – Slide Snapshot 400x Magnification

Lactobacillus delbrueckii subsp. *bulgaricus* cells measure 0.5-0.8 x 2.0-9.0 µm (Teixeira, 2014).
Streptococcus thermophilus cells measure 0.7 - 0.9 µm in diameter (Harnett, 2020).

Table 4
Methylene Blue Test - Cell Viability

Treatment	*% Viability*
Lucuma	78.37 ± 7.88[a]
Yacon	85.21 ± 5.15[a]
Monk Fruit	74.84 ± 8.12[a]
Control	84.80 ± 7.13[a]

Different superscripted letters in the same column indicate statistically significant difference among the values of the same parameter ($p < 0.05$).

± Standard deviations with respect to the mean values of triplicate runs.

4.3 Conclusions

This work dealt with an exploration of suitability of alternatives to replace sweeteners which have been shown to have a detrimental effect on probiotic bacteria. The following main conclusions may be drawn from these results. In culture medium, acidification kinetics were inhibited in the presence of sweeteners, thereby causing a deceleration in the maximum acidification rate, the time needed to reach v_{max}, and to complete fermentation. Viability was estimated from the methylene blue test and results were consistent with the acidification profile in that the control sample had more viable cells than the samples with added sweetener. These results may open the door to spark further exploration into microbiological analysis of sweeteners with the goal of reformulation and improvement of food products with health-promoting properties.

4.3.1 Limitations

The most pronounced limitation to this project was the sudden lockdown that resulted from the Covid-19 pandemic. Since the lab could not be accessed, experiments were cut short therefore it was not possible to continue our existing tests or set up any further trials. As a result there are inconclusive data and unexplored analysis which would have greatly enriched this work.

In order to isolate and enumerate colony numbers of lactic acid bacteria, the pour plate technique was employed by serial dilution of each culture and sweetener treatment in addition to a control; incubated anaerobically in triplicate according to standard procedures. Plate counts showed positive growth on an agar medium, however the colony counts were inconsistent with tenfold serial dilutions, rendering the tests inconclusive. If it was possible to get back into the lab, we would have engaged in additional viability analysis including additional rounds of plate counting.

Although it is still widely used, the methylene blue dyeing method is thought to overestimate viability, and there is high potential for human error (Evans, 2006). Flow cytometry is where cells are counted by passage through a laser beam, and this is a more reliable method of producing accurate viability determinations, however this apparatus was not available at our facility.

Isolated oligosaccharides have been the representative prebiotic agents in the majority of the current body of research. It should be taken into account that the yacon and lucuma used in this project are a commercial product and unrefined raw material, and there are currently no consistency standards for the production of the sweeteners. Consequently, it is possible the prebiotic fibres are not readily available to be metabolised by probiotic bacteria. Additionally, carbohydrate content in the crops could vary considerably based on factors such as cultivar variety, harvest time and location, and post harvest storage conditions. In yacon and lucuma, there are other components present that may have affected bacterial growth. The monk fruit employed in this project is in a concentrated form, and it is known to contain medicinal compounds which have antibacterial properties and have been shown to supress bacterial growth.

4.3.2 Future Research

Yacon and lucuma contain bioactive components that may have affected bacterial growth. Monk fruit is in a concentrated form and is known to contain medicinal compounds which have antibacterial properties and have been shown to supress bacterial growth. Any insights to better understand the precise compostion of the sweeteners would benefit this topic of research. Once these compounds are

identified then we could gain a better understanding of the mechanisms of inhibition components that impact the acidification process.

Future experiments could employ chromatographic techniques coupled with a sensitive detector such as gas chromatography-mass spectroscopy (GC-MS), which would be advantageous in order to measure the change in sugar composition in the milk and to analyse the metabolic products as they breakdown during fermentation.

It remains controversial whether a potential prebiotic agent must selectively stimulate only one or a limited number of probiotics, and a major emerging area of research is the focus on the specificity of prebiotics for gut bacteria (Wang *et al.*, 2020). Using variations in starter cultures in these experiments would be a insightful way to investigate growth dynamics and explore the selective stimulation of tailored prebiotics for the specific enrichment of gut probiotic populations at the strain level.

From a sensory point of view, the flavor profiles of yacon and lucuma show promise to enhance food product development and appeal to a wider population beyond the niche market. For instance, the natural essence of lucuma is a caramel butterscotch type flavour that would lend well to applications such as puddings, desserts, and dairy based treats. A sensorial component to this research area can assist in formulating with the sweeteners and attaining optimal sweetness potency, appearance, colour, texture, flavour, and overall acceptibility. An evaluation of the various physical, bioactive, and quality parameters, would greatly help to guide novel food innovation of products that will maintain a comparable sensory profile to sucrose sweetened foods without impacting consumer acceptability. Reformulation and new development using novel ingredients that can replace sucrose and create interesting flavours while also offering functional nutrition benefits, should be examined using tools such as the acceptibility test with 9-point hedonic scale, ranking and preference tests.

5 List of References

Abdel-Hamid, M., Romeih, E., Huang, Z., Enomoto, T., Huang, L. and Li, L. (2020) 'Bioactive properties of probiotic set-yogurt supplemented with Siraitia grosvenorii fruit extract', *Food Chemistry,* 303. doi: 10.1016/j.foodchem.2019.125400.

Abou-Donia, M., El-Masry, E., Abdel-Rahman, A., McLendon, R.E. and Schiffman, S.S. (2008) 'Splenda Alters Gut Microflora and Increases Intestinal P-Glycoprotein and Cytochrome P-450 in Male Rats', *Journal of Toxicology and Environmental Health, Part A;* 71(21), pp. 1415-1429. doi: 10.1080/15287390802328630.

Al-Goblan, A., Al-Alfi, M. and Khan, M.Z. (2014) 'Mechanism linking diabetes mellitus and obesity', *Diabetes, metabolic syndrome and obesity : targets and therapy,* 7, pp. 587-591. doi: 10.2147/DMSO.S67400.

Anton, S.D., Martin, C.K., Han, H., Coulon, S., Cefalu, W.T., Geiselman, P. and Williamson, D.A. (2010) 'Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels', *Appetite,* 55(1), pp. 37-43. doi: 10.1016/j.appet.2010.03.009.

Augustin, L.S.A., Kendall, C.W.C., Jenkins, D.J.A., Willett, W.C., Astrup, A., Barclay, A.W., Björck, I., Brand-Miller, J., Brighenti, F., Buyken, A.E., Ceriello, A., La Vecchia, C., Livesey, G., Liu, S., Riccardi, G., Rizkalla, S.W., Sievenpiper, J.L., Trichopoulou, A. and Wolever, T.M.S. (2015) 'Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC)', *Nutrition, Metabolism and Cardiovascular Diseases,* 25(9), pp. 795-815. doi: 10.1016/j.numecd.2015.05.005.

Baião, D.,Dos Santos, de Freitas, C.S., Gomes, L.P., Da Silva, D., Correa, Anna Carolina N. T. F., Pereira, P.R., Aguila, E.M.D. and Paschoalin, V.M.F. (2017) 'Polyphenols from Root, Tubercles and Grains Cropped in Brazil: Chemical and Nutritional Characterization and Their Effects on Human Health and Diseases', *Nutrients,* 9(9). doi: 10.3390/nu9091044.

Barba, F.J. (2019) *Innovative thermal and non-thermal processing, bioaccessibility and bioavailability of nutrients and bioactive compounds.* Duxford, England : Woodhead Publishing.

Belščak-Cvitanović, A., Komes, D., Dujmović, M., Karlović, S., Biškić, M., Brnčić, M. and Ježek, D. (2015) 'Physical, bioactive and sensory quality parameters of reduced sugar chocolates formulated with natural sweeteners as sucrose alternatives', *Food Chemistry,* 167, pp. 61-70. doi: 10.1016/j.foodchem.2014.06.064.

Bian, X., Chi, L., Gao, B., Tu, P., Ru, H. and Lu, K. (2017a) 'The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice', *PloS one,* 12(6), pp. e0178426. doi: 10.1371/journal.pone.0178426.

Bian, X., Chi, L., Gao, B., Tu, P., Ru, H. and Lu, K. (2017b) 'Gut Microbiome Response to Sucralose and Its Potential Role in Inducing Liver Inflammation in Mice', *Frontiers in physiology,* 8, pp. 487. doi: 10.3389/fphys.2017.00487.

Bian, X., Tu, P., Chi, L., Gao, B., Ru, H. and Lu, K. (2017c) 'Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions', *Food and chemical toxicology,* 107, pp. 530-539. doi: 10.1016/j.fct.2017.04.045.

Bibas Bonet, M.E., Meson, O., de Moreno de Leblanc, A, Dogi, C.A., Chaves, S., Kortsarz, A., Grau, A. and Perdigón, G. (2010) 'Prebiotic effect of yacon (Smallanthus sonchifolius) on intestinal mucosa using a mouse model', *Food and Agricultural Immunology,* 21(2), pp. 175-189. doi: 10.1080/09540100903563589.

Bintsis, T. (2018) 'Lactic acid bacteria as starter cultures: An update in their metabolism and genetics', *AIMS microbiology,* 4(4), pp. 665-684. doi: 10.3934/microbiol.2018.4.665.

Bohn, T. (2014) 'Dietary factors affecting polyphenol bioavailability', *Nutrition reviews,* 72(7), pp. 429-452. doi: 10.1111/nure.12114.

Bright, O.M., Wang, D.D., Shams-White, M., Bleich, S.N., Foreyt, J., Franz, M., Johnson, G., Manning, B.T., Mattes, R., Pi-Sunyer, X., Schneeman, B., Parrott, J.S., Steffen, D., Sylvetsky, A., Ziegler, P. and Chung, M. (2017) 'Research Priorities for Studies Linking Intake of Low-Calorie Sweeteners and Potentially Related Health Outcomes', *Current Developments in Nutrition,* 1(7). doi: 10.3945/cdn.117.000547.

Brizzolari, A., Brandolini, A., Glorio-Paulet, P. and Hidalgo, A. (2019) 'Antioxidant capacity and heat damage of powder products from South American plants with functional properties'*, Italian Journal of Food Science,* 31(4), pp. 731-748.

Brownawell, A.M., Caers, W., Gibson, G.R., Kendall, C.W.C., Lewis, K.D., Ringel, Y. and Slavin, J.L. (2012) 'Prebiotics and the health benefits of fiber: current regulatory status, future research, and goals', *The Journal of Nutrition,* 142(5), pp. 962-974. doi: 10.3945/jn.112.158147.

Bruyère, O., Ahmed, S., Atlan, C., Belegaud, J., Bortolotti, M., Charrière, S., Girardet, J., Houdart, S., Kalonji, E., Rajas, F., Slama, G. and Margaritis, I. (2015) 'Review of the nutritional benefits and risks related to intense sweeteners', *Archives of Public Health,* 73(1), pp. 1-10. doi: 10.1186/s13690-015-0092-x.

Campos, D., Betalleluz-Pallardel, I., Chirinos, R., Aguilar-Galvez, A., Noratto, G. and Pedreschi, R. (2012) 'Prebiotic effects of yacon (Smallanthus sonchifolius Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity', *Food Chemistry,* 135(3), pp. 1592-1599. doi: 10.1016/j.foodchem.2012.05.088.

Cao, Y., Zhang, H., Jin, Y., Zhang, Y. and Hayford, F. (2018) 'Phytochemical Properties and Nutrigenomic Implications of Yacon as a Potential Source of Prebiotic: Current Evidence and Future Directions', *Foods,* 7(4), pp. 59. doi: 10.3390/foods7040059.

Cerdó, T., García-Santos, J., Bermúdez, M.,G. and Campoy, C. (2019) 'The Role of Probiotics and Prebiotics in the Prevention and Treatment of Obesity', *Nutrients,* 11(3), pp. 635. doi: 10.3390/nu11030635.

Chandrasekara and Kumar (2016) 'Roots and Tuber Crops as Functional Foods: A Review on Phytochemical Constituents and Their Potential Health Benefits', *International Journal of Food Science,* 2016. doi: 10.1155/2016/3631647.

Choque Delgado, G., da Silva Cunha Tamashiro, Wirla, Maróstica Junior, M. and Pastore, G. (2013) 'Yacon (Smallanthus sonchifolius): A Functional Food', *Plant Foods for Human Nutrition,* 68(3), pp. 222-228. doi: 10.1007/s11130-013-0362-0.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J. and Turnbaugh, P.J. (2014) 'Diet rapidly and reproducibly alters the human gut microbiome', *Nature,* 505(7484), pp. 559-563. doi: 10.1038/nature12820.

de Almeida Paula, H. and Abranches, M. (2015) 'Yacon (Smallanthus Sonchifolius): A Food with Multiple Functions', *Critical reviews in food science and nutrition,* 55(1), pp. 32-40. doi: 10.1080/10408398.2011.645259.

De Souza Oliveira, Perego, P., Converti, A., De Oliveira, M.N. and Pinheiro, R. (2009) 'Growth and acidification performance of probiotics in pure culture and co-culture with Streptococcus thermophilus: The effect of inulin', *LWT - Food Science and Technology,* 42(5), pp. 1015-1021. doi: 10.1016/j.lwt.2009.01.002.

De Vrese, M. and Schrezenmeir, J. (2008) 'Probiotics, prebiotics, and synbiotics', *Advances in Biochemical Engineering/Biotechnology,* 111, pp. 1-66. doi: 10.1007/10_2008_097.

Dini, I. (2011) 'Flavonoid glycosides from Pouteria obovata (R. Br.) fruit flour', *Food Chemistry,* 124(3), pp. 884-888. doi: 10.1016/j.foodchem.2010.07.013.

Donaldson, G.P., Lee, S.M. and Mazmanian, S.K. (2015) 'Gut biogeography of the bacterial microbiota', *Nature reviews. Microbiology,* 14(1), pp. 20-32. doi: 10.1038/nrmicro3552.

Duarte, O. and Paull, R.E. (2015) *Exotic Fruits and Nuts of the New World.* CABI.

Esmerino, E.A., Cruz, A.G., Pereira, E.P.R., Rodrigues, J.B., Faria, J.A.F. and Bolini, H.M.A. (2013) 'The influence of sweeteners in probiotic Petit Suisse cheese in concentrations equivalent to that of sucrose', *Journal of dairy science,* 96(9), pp. 5512-5521. doi: https://doi.org/10.3168/jds.2013-6616.

Evans, E. (2006) *Brewing: science and practice,* Chichester, UK: John Wiley & Sons, Ltd.

Farnworth, E.R. (2003) *Handbook of fermented functional foods*, Boca Raton, Fla: CRC Press.

Feijó, F.,de Matos, Ballard, C., Foletto, K.C., Batista, B.A.M., Neves, A.M., Ribeiro, M.F.M. and Bertoluci, M.C. (2013) 'Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels', *Appetite,* 60, pp. 203-207.

Fitch, C. and Keim, K.S. (2012) 'Position of the Academy of Nutrition and Dietetics: Use of Nutritive and Nonnutritive Sweeteners', *Journal of the Academy of Nutrition and Dietetics,* 112(5), pp. 739. doi: 10.1016/j.jand.2012.03.009.

Fowler, S.P., Williams, K., Resendez, R.G., Hunt, K.J., Hazuda, H.P. and Stern, M.P. (2008) 'Fueling the Obesity Epidemic? Artificially Sweetened Beverage Use and Long-term Weight Gain', *Obesity,* 16(8), pp. 1894-1900. doi: 10.1038/oby.2008.284.

Fuentealba, C., Gálvez, L., Cobos, A., Olaeta, J.A., Defilippi, B.G., Chirinos, R., Campos, D. and Pedreschi, R. (2016) 'Characterization of main primary and secondary metabolites and in vitro antioxidant and antihyperglycemic properties in the mesocarp of three biotypes of Pouteria lucuma', *Food Chemistry,* 190, pp. 403-411. doi: 10.1016/j.foodchem.2015.05.111.

García-Ríos, D., Aguilar-Galvez, A., Chirinos, R., Pedreschi, R. and Campos, D. (2020) 'Relevant physicochemical properties and metabolites with functional properties of two commercial varieties of Peruvian Pouteria lucuma', *Journal of Food Processing and Preservation,* 44(6), pp. n/a. doi: 10.1111/jfpp.14479.

Gardner, C., Wylie-Rosett, J., Gidding, S.S., Steffen, L.M., Johnson, R.K., Reader, D. and Lichtenstein, A.H. (2012) 'Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association.(SCIENTIFIC STATEMENT)', *Diabetes care,* 35(8), pp. 1798. doi: 10.2337/dc12-9002.

Ghosh, S. and Collier, A. (2012) *Churchill's Pocketbook of Diabetes: Second Edition,* pp. 83-125. Churchill Livingstone.

Gibson, G.R., Hutkins, R., Mary, E.S., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K. and Reid, G. (2017) 'Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics', *Nature Reviews Gastroenterology & Hepatology,* . doi: 10.1038/nrgastro.2017.75.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A. and Roberfroid, M.B. (2004) 'Dietary modulation of the human colonic microbiota: updating the concept of prebiotics', *Nutrition research reviews,* 17(2), pp. 259. doi: 10.1079/NRR200479.

Gibson, G.R. and Roberfroid, M.B. (1995) 'Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics', *The Journal of nutrition,* 125(6), pp. 1401-1412. doi: 10.1093/jn/125.6.1401.

Glorio, P., Repo-Carrasco, R., Velezmoro, C., Anticona, S., Huaranga, R., Martínez, P., Melgarejo, S., Astuhuaman, L., Huamán, N.E., Icochea, J.C. and Peña, J.C. (2008) 'Fibra dietaria en variedades peruanas de frutas, tubérculos, cereales y leguminosas', *Revista de la Sociedad Química del Perú,* 74(1), pp. 46-56.

Gomes Da Silva, Dionísio, A.P., Ferreira Carioca, A.A., Silveira Adriano, L., Pinto, C.O., Pinto de Abreu, Fernando Antonio, Wurlitzer, N.J., Araújo, I.M., Dos Santos Garruti, D. and Ferreira Pontes, D. (2017) 'Yacon syrup: Food applications and impact on satiety in healthy volunteers', *Food Research International,* 100, pp. 460-467. doi: 10.1016/j.foodres.2017.07.035.

Gómez-Maqueo, A., Bandino, E., Hormaza, J.I. and Cano, M.P. (2020) 'Characterization and the impact of in vitro simulated digestion on the stability and bioaccessibility of carotenoids and their esters in two Pouteria lucuma varieties', *Food Chemistry,* 316.

Gong, X., Chen, N., Ren, K., Jia, J., Wei, K., Zhang, L., Lv, Y., Wang, J. and Li, M. (2019) 'The Fruits of Siraitia grosvenorii: A Review of a Chinese Food-Medicine', *Frontiers in pharmacology,* 10, pp. 1400. doi: 10.3389/fphar.2019.01400.

Graefe, S., Hermann, M., Manrique, I., Golombek, S. and Buerkert, A. (2004) 'Effects of post-harvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes', *Field Crops Research,* 86(2), pp. 157-165. doi: 10.1016/j.fcr.2003.08.003.

Harnett, J., Patrick, A., Caddick, C., Pearce, L. and Davey, G. (2020) 'Streptococcus thermophilus'. *Reference Module in Food Science.* Elsevier.

Hermann, M. (2009) 'The impact of the European Novel Food Regulation on trade and food innovation based on traditional plant foods from developing countries', *Food Policy,* 34(6), pp. 499-507. doi: 10.1016/j.foodpol.2009.08.005.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C. and Sanders, M.E. (2014) 'The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic', *Nature Reviews Gastroenterology & Hepatology,* 11(8), pp. 506-514. doi: 10.1038/nrgastro.2014.66.

Hollister, E.B., Gao, C. and Versalovic, J. (2014) 'Compositional and functional features of the gastrointestinal microbiome and their effects on human health', *Gastroenterology,* 146(6), pp. 1449-1458. doi: 10.1053/j.gastro.2014.01.052.

Holzapfel (1995) *The Genera of Lactic Acid Bacteria.* 1st edition, New York, NY : Springer US.

Holzapfel and Schillinger, U. (2002) 'Introduction to pre- and probiotics', *Food Research International,* 35(2-3), pp. 109-116. doi: 10.1016/S0963-9969(01)00171-5.

Hungin, A.P.S., Mitchell, C.R., Whorwell, P., Mulligan, C., Cole, O., Agréus, L., Fracasso, P., Lionis, C., Mendive, J., Philippart De Foy, J. -M., Seifert, B., Wensaas, K.-, Winchester, C. and Wit, N. (2018) 'Systematic review: probiotics in the management of lower gastrointestinal symptoms – an updated evidence-based international consensus', *Alimentary Pharmacology & Therapeutics,* 47(8), pp. 1054-1070. doi: 10.1111/apt.14539.

Huppertz, T., Fox, P.F. and Kelly, A.L. (2018) The caseins: Structure, stability, and functionality, *Proteins in Food Processing* (Second edition, pp. 49-92). Elsevier Ltd.

Imamura, F., O'Connor, L. and Forouhi, N. (2015) 'Positive association between artificially sweetened beverage consumption and incidence of diabetes. Reply to Sylvetsky Meni AC, Swithers SE, Rother KI [letter]', *Diabetologia; Clinical and Experimental Diabetes and Metabolism,* 58(10), pp. 2457-2458. doi: 10.1007/s00125-015-3706-5.

Itkin, M., Davidovich-Rikanati, R., Cohen, S., Portnoy, V., Doron-Faigenboim, A., Oren, E., Freilich, S., Tzuri, G., Baranes, N., Shen, S., Petreikov, M., Sertchook, R., Ben-Dor, S., Gottlieb, H., Hernandez, A., Nelson, D.R., Paris, H.S., Tadmor, Y., Burger, Y., Lewinsohn, E., Katzir, N. and Schaffer, A. (2016) 'The biosynthetic pathway of the nonsugar, high-intensity sweetener mogroside V from Siraitia grosvenorii', *Proceedings of the National Academy of Sciences of the United States of America,* 113(47), pp. E7619. doi: 10.1073/pnas.1604828113.

Jin, J. and Lee, J. (2012) 'Phytochemical and pharmacological aspects of Siraitia grosvenorii, luo han kuo'. *Oriental Pharmacy and Experimental Medicine, 12*(4), pp. 233-239.

Kroger, M., Meister, K. and Kava, R. (2006) 'Low-calorie Sweeteners and Other Sugar Substitutes: A Review of the Safety Issues', *Comprehensive Reviews in Food Science and Food Safety,* 5(2), pp. 35-47. doi: 10.1111/j.1541-4337.2006.tb00081.x.

Kumar and Pandey, A.K. (2013) 'Chemistry and Biological Activities of Flavonoids: An Overview', *The Scientific World Journal,* 2013. doi: 10.1155/2013/162750.

Kumar, Salminen, S., Verhagen, H., Rowland, I., Heimbach, J., Bañares, S., Young, T., Nomoto, K. and Lalonde, M. (2015) 'Novel probiotics and prebiotics: road to the market', *Current Opinion in Biotechnology,* 32, pp. 99-103. doi: 10.1016/j.copbio.2014.11.021.

Laudisi, Stolfi and Monteleone (2019) 'Impact of Food Additives on Gut Homeostasis', *Nutrients,* 11(10), pp. 2334. doi: 10.3390/nu11102334.

Lee, Y.K. and Salminen, S. (2009) *Handbook of Probiotics and Prebiotics.* Second edition, Hoboken, N.J.: John Wiley & Sons, Inc.

Leroy, F. and De Vuyst, L. (2004) 'Lactic acid bacteria as functional starter cultures for the food fermentation industry'. *Trends in Food Science & Technology,* 15(2), pp. 67-78.

Ley, R.E., Fredrik Bäckhed, Turnbaugh, P., Lozupone, C.A., Knight, R.D. and Gordon, J.I. (2005) 'Obesity alters gut microbial ecology', *Proceedings of the National Academy of Sciences of the United States of America,* 102(31), pp. 11070. doi: 10.1073/pnas.0504978102.

Ley, R.E., Turnbaugh, P.J., Klein, S. and Gordon, J.I. (2006) 'Microbial ecology: Human gut microbes associated with obesity', *Nature,* 444(7122), pp. 1022. doi: 10.1038/4441022a.

Li, C., Lin, L., Sui, F., Wang, Z., Huo, H., Dai, L. and Jiang, T. (2014) 'Chemistry and pharmacology of Siraitia grosvenorii: A review'. *Chinese Journal of Natural Medicines,* 12(2), pp. 89-102.

Lin, T.Y. (2000) 'Conjugated linoleic acid concentration as affected by lactic cultures and additives', *Food Chemistry,* 69(1), pp. 27-31. doi: 10.1016/S0308-8146(99)00218-6.

Lu, F., Li, D., Fu, C., Liu, J., Huang, Y., Chen, Y., Wen, Y. and Nohara, T. (2012) 'Studies on chemical fingerprints of Siraitia grosvenorii fruits (Luo Han Guo) by HPLC', *Journal of Natural Medicines,* 66(1), pp. 70-76. doi: 10.1007/s11418-011-0555-5.

Lucey, J.A. (2004) 'Cultured dairy products: an overview of their gelation and texture properties', *International Journal of Dairy Technology,* 57(2-3), pp. 77-84. doi: 10.1111/j.1471-0307.2004.00142.x.

Ma, J., Yang, H., Basile, M.J. and Kennelly, E.J. (2004) 'Analysis of polyphenolic antioxidants from the fruits of three pouteria species by selected ion monitoring liquid chromatography-mass spectrometry', *Journal of Agricultural and Food Chemistry,* 52(19), pp. 5873-5878. doi: 10.1021/jf049950k.

Macgregor, G.A. and Hashem, K.M. (2014) 'Action on sugar—lessons from UK salt reduction programme', *The Lancet,* 383(9921), pp. 929-931. doi: 10.1016/S0140-6736(14)60200-2.

Magnuson, B.A., Carakostas, M.C., Moore, N.H., Poulos, S.P. and Renwick, A.G. (2016) 'Biological fate of low-calorie sweeteners', *Nutrition reviews,* 74(11), pp. 670-689. doi: 10.1093/nutrit/nuw032.

Manning, T.S. and Gibson, G.R. (2004) 'Prebiotics', *Best Practice & Research Clinical Gastroenterology,* 18(2), pp. 287-298. doi: 10.1016/j.bpg.2003.10.008.

Manrique, I. (2005) *Yacon syrup: Principles and proccessing.* Lima, Perú : International Potato Center.

Markowiak, P. and Śliżewska, K. (2017) 'Effects of Probiotics, Prebiotics, and Synbiotics on Human Health', *Nutrients,* 9(9), pp. 1021. doi: 10.3390/nu9091021.

Mérillon, J. and Ramawat, K.G. (2018) *Sweeteners Pharmacology, Biotechnology, and Applications.* 1st edition, Springer International Publishing.

Migliore, G., Borrello, M., Lombardi, A. and Schifani, G. (2018) *Consumers' willingness to pay for natural food: evidence from an artefactual field experiment*, Berlin/Heidelberg: Springer Science and Business Media LLC.

Mohammadi, R. and Mortazavian, A.M. (2011) 'Review Article: Technological Aspects of Prebiotics in Probiotic Fermented Milks', *Food Reviews International,* 27(2), pp. 192-212. doi: 10.1080/87559129.2010.535235.

Narvhus, J.A. and Axelsson, L. (2003) *Lactic Acid Bacteria*, Oxford: Academic Press.

National Research Council (2000) *Lost Crops of the Incas: Little-Known Plants of the Andes with Promise for Worldwide Cultivation.* National Academies Press.

Nichol, A.D., Salame, C., Rother, K.I. and Pepino, M.Y. (2019) 'Effects of Sucralose Ingestion versus Sucralose Taste on Metabolic Responses to an Oral Glucose Tolerance Test in Participants with Normal Weight and Obesity: A Randomized Crossover Trial', *Nutrients,* 12(1). doi: 10.3390/nu12010029.

Ojansivu, I., Ferreira, C.L. and Salminen, S. (2011) 'Yacon, a new source of prebiotic oligosaccharides with a history of safe use', *Trends in Food Science & Technology,* 22(1), pp. 40-46. doi: 10.1016/j.tifs.2010.11.005.

Oliveira, R., Florence, A., Silva, R.C., Perego, P., Converti, A., Gioielli, L.A. and Oliveira, M.N. (2009) 'Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk', *International Journal of Food Microbiology,* 128(3), pp. 467-472. doi: 10.1016/j.ijfoodmicro.2008.10.012.

Padilha, V.M., Andrade, S.A.C., Valencia, M.S., Stamford, T. and Salgado, S.M. (2017) 'Optimization of synbiotic yogurts with yacon pulp (Smallanthus sonchifolius) and assessment of the viability of lactic acid bacteria', *Food Science and Technology,* 37(2), pp. 166-175. doi: 10.1590/1678-457x.14016.

Palmnäs, M.S.A., Cowan, T.E., Bomhof, M.R., Su, J., Reimer, R.A., Vogel, H.J., Hittel, D.S., Shearer, J. and Müller, M. (2014) 'Low-Dose Aspartame Consumption Differentially Affects Gut Microbiota-Host Metabolic Interactions in the Diet-Induced Obese Rat', *PLoS ONE,* 9(10). doi: 10.1371/journal.pone.0109841.

Pandey, A.K. and Chauhan, O.P. (2019) 'Monk fruit (Siraitia grosvenorii) - health aspects and food applications', *Pantnagar Journal of Research,* 17(3).

Pei, R., Liu, X. and Bolling, B. (2020) 'Flavonoids and gut health', *Current opinion in biotechnology,* 61, pp. 153-159. doi: 10.1016/j.copbio.2019.12.018.

Piccolella, S. and Pacifico, S. (2015) 'Plant-Derived Polyphenols: A Chemopreventive and Chemoprotectant Worth-Exploring Resource in Toxicology', *Advances in Molecular Toxicology*, pp. 161-214.

Qi, X., Chen, W., Zhang, L. and Xie, B. (2008) 'Mogrosides extract from Siraitia grosvenori scavenges free radicals in vitro and lowers oxidative stress, serum glucose, and lipid levels in alloxan-induced diabetic mice', *Nutrition Research,* 28(4), pp. 278-284.

Quinlan, M.E. and Zhou, Y. (2017) *Sweetener Compositions Containing Monk Fruit Extract and Rebaudiosides A and B.* Authoring organisation. US9609887B2. Available at: https://patentimages.storage.googleapis.com/2e/c1/8b/e2b586237c5b85/US9609887.pdf

Ribas-Agustí, A., Martín-Belloso, O., Soliva-Fortuny, R. and Elez-Martínez, P. (2018) 'Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods', *Critical Reviews in Food Science and Nutrition*, 58(15), pp. 2531-2548.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P.I., Godneva, A., Kalka, I.N., Bar, N., Shilo, S., Lador, D., Arnau, V.V., Zmora, N., Meirav Pevsner-Fischer, Israeli, D., Kosower, N., Malka, G., Bat, C.W., Tali Avnit-Sagi, Maya Lotan-Pompan, Weinberger, A., Halpern, Z., Carmi, S., Fu, J., Wijmenga, C., Zhernakova, A., Elinav, E. and Segal, E. (2018) 'Environment dominates over host genetics in shaping human gut microbiota', *Nature,* 555(7695), pp. 210. doi: 10.1038/nature25973.

Ruiz-Ojeda, F., Plaza-Díaz, J., Sáez-Lara, M.J. and Gil, A. (2019) 'Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials', *Advances in Nutrition,* 10, pp. S31-S48. doi: 10.1093/advances/nmy037.

Sanders, M.E., Merenstein, D., Merrifield, C.A. and Hutkins, R. (2018) 'Probiotics for human use', *Nutrition Bulletin,* 43(3), pp. 212-225. doi: 10.1111/nbu.12334.

Saura-Calixto, F. (2011) 'Dietary fiber as a carrier of dietary antioxidants: an essential physiological function', *Journal of Agricultural and Food Chemistry,* 59(1), pp. 43-49. doi: 10.1021/jf1036596.

Senghor, B., Sokhna, C., Ruimy, R. and Lagier, J. (2018) 'Gut microbiota diversity according to dietary habits and geographical provenance', *Human Microbiome Journal,* 7-8, pp. 1-9. doi: 10.1016/j.humic.2018.01.001.

Sergeev, I., Aljutaily, T., Walton, G. and Huarte, E. (2020) 'Effects of Synbiotic Supplement on Human Gut Microbiota, Body Composition and Weight Loss in Obesity', *Nutrients,* 12(1), pp. 222. doi: 10.3390/nu12010222.

Shankar, P., Ahuja, S. and Sriram, K. (2013) 'Non-nutritive sweeteners: Review and update', *Nutrition*, 29(11), pp. 1293-1299.

Sheard, N.F., Clark, N.G., Brand-Miller, J., Franz, M.J., Pi-Sunyer, F., Mayer-Davis, E., Kulkarni, K. and Geil, P. (2004) 'Dietary carbohydrate (amount and type) in the prevention and management of diabetes: a statement by the American Diabetes Association.(Reviews/Commentaries/ADA Statements)', *Diabetes Care,* 27(9), pp. 2266.

Shori, A.B. (2016) 'Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages', *Food Bioscience,* 13, pp. 1-8. doi: 10.1016/j.fbio.2015.11.001.

Sieuwerts, S. (2016) 'Microbial Interactions in the Yoghurt Consortium: Current Status and Product Implications', *SOJ Microbiol Infect Dis,* 4(2).

Silva, C., Simeoni, L.A. and Silveira, D. (2009) 'Genus Pouteria: chemistry and biological activity', *Revista Brasileira de Farmacognosia,* 19(2), pp. 501-509. doi: 10.1590/S0102-695X2009000300025.

Singh, R., Chang, H., Yan, D., Lee, K., Ucmak, D., Wong, K., Abrouk, M., Farahnik, B., Nakamura, M., Zhu, T., Bhutani, T. and Liao, W. (2017) 'Influence of diet on the gut microbiome and implications for human health', *Journal Of Translational Medicine,* 15(1). doi: 10.1186/s12967-017-1175-y.

Slavin, J. (2013) 'Fiber and Prebiotics: Mechanisms and Health Benefits', *Nutrients,* 5(4), pp. 1417-1435. doi: 10.3390/nu5041417.

Sonnenburg, J.L. and Bäckhed, F. (2016) 'Diet–microbiota interactions as moderators of human metabolism', *Nature,* 535(7610), pp. 56. doi: 10.1038/nature18846.

Suez, J., Korem, T., Zeevi, D., Gili Zilberman-Schapira, Thaiss, C.A., Maza, O., Israeli, D., Zmora, N., Gilad, S., Weinberger, A., Kuperman, Y., Harmelin, A., Ilana Kolodkin-Gal, Shapiro, H., Halpern, Z., Segal, E. and Elinav, E. (2014) 'Artificial sweeteners induce glucose intolerance by altering the gut microbiota', *Nature,* 514(7521). doi: 10.1038/nature13793.

Suez, J., Korem, T., Zilberman-Schapira, G., Segal, E. and Elinav, E. (2015) 'Non-caloric artificial sweeteners and the microbiome: findings and challenges', *Gut Microbes,* 6(2), pp. 149-155. doi: 10.1080/19490976.2015.1017700.

Suzuki, Y.A., Tomoda, M., Murata, Y., Inui, H., Sugiura, M. and Nakano, Y. (2007) 'Antidiabetic effect of long-term supplementation with Siraitia grosvenori on the spontaneously diabetic Goto–Kakizaki rat', *British Journal of Nutrition*, 97(4), pp. 770-775.

Sylvetsky, A.C. (2018) 'Metabolic Effects of Low-Calorie Sweeteners: A Brief Review', *Obesity,* 26, pp. S25-S31. doi: 10.1002/oby.22252.

Sylvetsky, A.C. and Rother, K.I. (2018) 'Nonnutritive Sweeteners in Weight Management and Chronic Disease: A Review', *Obesity*, 26(4), pp. 635-640.

Takasaki, M., Konoshima, T., Murata, Y., Sugiura, M., Nishino, H., Tokuda, H., Matsumoto, K., Kasai, R. and Yamasaki, K. (2003) 'Anticarcinogenic activity of natural sweeteners, cucurbitane glycosides, from Momordica grosvenori', *Cancer letters,* 198(1), pp. 37-42. doi: 10.1016/s0304-3835(03)00285-4.

Teixeira, P. (2014) 'LACTOBACILLUS | Lactobacillus delbrueckii ssp. bulgaricus', in Batt, C.A. and Tortorello, M.L. (eds.) *Encyclopedia of Food Microbiology (Second Edition)* Oxford: Academic Press, pp. 425-431.

Terpou, A., Papadaki, A., Lappa, I., Kachrimanidou, V., Bosnea, L. and Kopsahelis, N. (2019) 'Probiotics in Food Systems: Significance and Emerging Strategies Towards Improved Viability and Delivery of Enhanced Beneficial Value', *Nutrients,* 11(7). doi: 10.3390/nu11071591.

Thursby, E. and Juge, N. (2017) 'Introduction to the human gut microbiota', *The Biochemical Journal,* 474(11), pp. 1823-1836. doi: 10.1042/BCJ20160510.

Topping, D.L. and Clifton, P.M. (2001) 'Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides', *Physiological Reviews,* 81(3), pp. 1031-1064. doi: 10.1152/physrev.2001.81.3.1031.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R. and Gordon, J.I. (2006) 'An obesity-associated gut microbiome with increased capacity for energy harvest', *Nature,* 444(7122), pp. 1027. doi: 10.1038/nature05414.

USDA (2017) *U. S. GRAS Notice 706*.

Valdes, A.M., Walter, J., Segal, E. and Spector, T.D. (2018) 'Role of the gut microbiota in nutrition and health', *British Medical Journal Publishing Group*, pp. 36.

Vamanu, E., Pelinescu, D., Gatea, F. and Sârbu, I. (2019) 'Altered in Vitro Metabolomic Response of the Human Microbiota to Sweeteners', *Genes,* 10(7), pp. 535. doi: 10.3390/genes10070535.

van Gunst, A. and Roodenburg, A. (2019) 'Consumer Distrust about E-numbers: A Qualitative Study among Food Experts', *Foods,* 8(5). doi: 10.3390/foods8050178.

Vinderola, C.G., Costa, G.A., Regenhardt, S. and Reinheimer, J.A. (2002) 'Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria', *International Dairy Journal,* 12(7), pp. 579-589. doi: 10.1016/S0958-6946(02)00046-8.

Wang (2009) 'Prebiotics: Present and future in food science and technology', *Food Research International,* 42(1), pp. 8-12.

Wang, B., Yang, Z., Xin, Z., Ma, G., Qian, Y., Xie, T. and Prakash, I. (2019a) 'Analysis of Mogrosides in Siraitia grosvenorii Fruits at Different Stages of Maturity', *Natural Product Communications,* 14(9). doi: 10.1177/1934578X19878621.

Wang, S., Xiao, Y., Tian, F., Zhao, J., Zhang, H., Zhai, Q. and Chen, W. (2020) 'Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms', *Journal of Functional Foods,* 66. doi: 10.1016/j.jff.2020.103838.

Wang, X., Guo, J., Liu, Y., Yu, H. and Qin, X. (2019b) 'Sucralose Increased Susceptibility to Colitis in Rats', *Inflammatory Bowel Diseases,* 25(2), pp. e3-e4. doi: 10.1093/ibd/izy196.

Wang, Browman, D., Herzog, H. and G, G.N. (2018) 'Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice', *PLoS ONE,* 13(7), pp. e0199080. doi: 10.1371/journal.pone.0199080.

Wang, Yao, M., Lv, L., Ling, Z. and Li, L. (2017) 'The Human Microbiota in Health and Disease', *Engineering,* 3(1), pp. 71-82. doi: 10.1016/J.ENG.2017.01.008.

Wedajo, B. (2015) 'Lactic Acid Bacteria: Benefits, Selection Criteria and Probiotic Potential in Fermented Food', *Journal of Probiotics & Health,* 03(02). doi: 10.4172/2329-8901.1000129.

Wiebe, N., Padwal, R., Field, C., Marks, S., Jacobs, R. and Tonelli, M. (2011) 'A systematic review on the effect of sweeteners on glycemic response and clinically relevant outcomes', *Bmc Medicine,* 9(1), pp. 123. doi: 10.1186/1741-7015-9-123.

Wu, G., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. and Lewis, J.D. (2011) 'Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes', *Science,* 334(6052), pp. 105-108. doi: 10.1126/science.1208344.

Yahia, E.M. and Guttierrez-Orozco, F. (2011) *Postharvest Biology and Technology of Tropical and Subtropical Fruits.* Elsevier Science & Technology.

Yan, M.R., Welch, R., Rush, E.C., Xiang, X. and Wang, X. (2019) 'A Sustainable Wholesome Foodstuff; Health Effects and Potential Dietotherapy Applications of Yacon', *Nutrients,* 11(11). doi: 10.3390/nu11112632.

Younes, M., Aquilina, G., Engel, K., Fowler, P., Frutos Fernandez, M.J., Fürst, P., Gürtler, R., Gundert-Remy, U., Husøy, T., Mennes, W., Moldeus, P., Oskarsson, A., Shah, R. and Waalkens-Berendsen (2019) 'Safety of use of Monk fruit extract as a food additive in different food categories', *EFSA Journal,* 17(12). doi: 10.2903/j.efsa.2019.5921.

Zhang, Yang, H., Zhang, H., Wang, Y. and Hu, P. (2011) 'Development of a Process for Separation of Mogroside V from Siraitia grosvenorii by Macroporous Resins', *Molecules,* 16(9), pp. 7288-7301. doi: 10.3390/molecules16097288.

Zhang, Yang, Y., Liang, Y., Jiao, X. and Zhao, C. (2018) 'Beneficial Effect of Intestinal Fermentation of Natural Polysaccharides', *Nutrients,* 10(8). doi: 10.3390/nu10081055.

6 Appendices

Ethical Approval Form